"FORCED DEGRADATION STUDIES OF ALVERINE CITRATE IN BULK AND FORMULATION BY UV, IR SPECTROPHOTOMETRY, TLC, AND RP-HPLC METHOD"

A dissertation submitted to

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Chennai-600032

In partial fulfillment of the requirements

for the award of the degree of

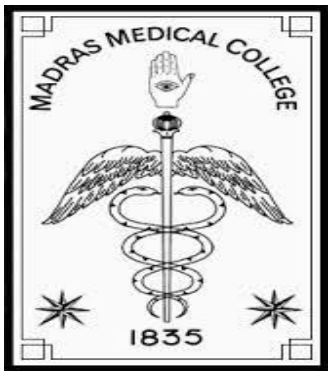
MASTER OF PHARMACY

IN

PHARMACEUTICAL CHEMISTRY

Submitted by

Reg. No.261215702



DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE

CHENNAI-600003

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CERTIFICATE

This is to certify that the dissertation entitled **"FORCED DEGRADATION STUDIES OF STUDIES OF ALVERINE CITRATE IN BULK AND FORMULATION BY UV, IR SPECTROPHOTOMETRY, TLC, AND RP-HPLCMETHOD"** is submitted by the candidate bearing the register no **261215702** in partial fulfillment of the requirements for the award of degree in **MASTER OF PHARMACY IN PHARMACEUTICAL CHEMISTRY** by the Tamil Nadu Dr. M.G.R Medical University, Chennai, is a bonafide work done by him during the academic year **2012-2014** at the **Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai 03.**

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LIST OF ABBREVIATIONS

S.No	ABBREVIATION	EXPANSION
1.	%	Percentage
2.	µg	Microgram
3.	µl	Microliter
4.	Abs	Absorbance
5.	API	Active Pharmaceutical Ingredient
6.	Avg.	Average
7.	Cm	Centimeter
8.	Dil.	Dilution
9.	G	Gram
10.	H_2O_2	Hydrogen Peroxide
11.	HCl	Hydrochloric Acid
12.	HPLC	High Performance Liquid Chromatography
13.	IR	Infrared
14.	KBr	Potassium Bromide
15.	Mg	Milligram
16.	Mins	Minutes
17.	Ml	Milliliter
18.	NaOH	Sodium Hydroxide
19.	Nm	Nanometer
20.	º	Celsius
21.	Rf	Retention Factor
22.	Sam	Sample
23.	Std	Standard
24.	UV	Ultra Violet
25.	Vol	Volume
26.	Wt.	Weight
27.	λ	Lambda

CONTENTS

INTRODUCTION

Analytical chemistry is often described as the area of chemistry responsible for characterizing the composition of matter, both qualitatively (what is present) and quantitatively (how much is present). Analytical chemistry is not a separate branch of chemistry, but simply the application of chemical knowledge. **(David Harvey 1997)**

Pharmaceutical Analysis is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and quantitative measurements of the substances present in bulk and pharmaceutical preparation. **(Sharma BK 2000)**

Quantitative analysis constitutes the largest part of analytical chemistry and is related to the various methods and instrumentation employed in determining the amounts or concentration of constituents in samples. It is also one of the basic criteria in the field of pharmacy where quality is to be critically maintained. Analytical chemistry may be defined as the "Science and art of determining the composition of materials in terms of the elements or compounds contained". Analytical method is a specific application of a technique to solve an analytical problem**. (Michael E *et al.,*)**

1. DEGRADATION STUDIES

A degradation product is defined as a chemical change in the drug molecule brought about over time and/or by action of, e.g., light, temperature, pH, or water or by reaction with an excipient and/or the immediate container/closure system (also called decomposition product). As per ICH guideline Stress testing helps determine the intrinsic stability of the molecule by establishing degradation pathways in order to identify the likely degradation products and to validate the stability indicating power of the analytical procedures used. Stress testing is conducted to provide data on forced decomposition products and decomposition mechanisms. The severe conditions that may be encountered during distribution can be covered by stress testing. These studies should stabilize the inherent stability characteristics of the molecule, such as the degradation pathways, and lead to identification of the degradation products and hence support the suitability of the

proposed analytical procedures. The detailed nature of the studies will depend on the individual drug substance and type of drug product. (**Monika Bakshi *et al.,*(2002**)

The studies include the following

- Development and validation of stability indicating method.
- Determination of degradation pathways of drug substance and drug product.
- Identification of degraded products in formulation that are related to drug substance versus those that are related to non-drug substance (Additives, Excipients)
- Structural elucidation of degraded fragment.
- Determination of intrinsic stability of drug substances.

2. IMPURITY STUDIES

Impurities in a drug substance (a new chemical entity of therapeutic interest) or drug product (a drug substance formulated into a suitable product for administration to patient) may cause serious side effects and hence we need to isolate and characterize impurities. **(FDA Guidance for Industry 2000)**

The following definition of impurity is currently under consideration by the regulatory bodies, which is likely to be included in the future guidance:

Impurity: any entity of the drug substance (bulk material) or drug product (final container product) that is not the chemical entity defined as the drug substance, an excipient, or other additives to the drug product.

Related substances: These substances are structurally related to the drug substance and may be identified or unidentified degradation products or impurities arising from a manufacturing process or during storage of a material.

As per ICH guidelines Impurities are listed alphabetically as given below.

- By-product
- Degradation product
- Interaction product
- Intermediate
- Penultimate intermediate
- Related product
- Transformation product

By-products: The unplanned compounds generated in the reaction to produce API are generally called by-products. For example during the synthesis of paracetamol we get diacetylated paracetamol which is the unexpected product called by-product.

Degradation products: The compounds produced as a result of decomposition of the material of interest or API is often called degradation products. Due to degradation, impurities may also be formed. Degradation of beta lactum ring in penicillin and cephalosporin are the examples of degradation products.

Related products: As suggested previously, the term "related products" tends to imply that the impurity is similar to the drug substance, and it thus tends to downplay the negativity frequently attached to the term "Impurity". Transformation products: This is relatively a term that relates to theorized and non-theorized products that may be produced in the reaction.

1.1. Classification of impurities

According to ICH guidelines, impurities can be broadly classified in to three categories for the drug substance produced by chemical synthesis:

- Organic impurities (starting materials, process-related products, inter- mediators, and degradation products).
- Inorganic impurities (salts, catalysts, ligands, and heavy metals or other residual metals).

- Residual solvents (organic and inorganic liquids used during production and/or recrystallization).

1.2. Need to Isolate and Characterize Impurities

Impurities are generally assumed to be inferior to API because they might not have the same level of pharmacologic activity. However, they are not necessarily always inferior. From the standpoint of its usage, the drug substance is compromised in terms of purity even if it contains another material with superior pharmacological or toxicological properties. At first pass this may not be readily apparent; however, on further thought it will become clear that if we are to ensure that the accurate amount of the drug substance is being administered to the patient, we must assess its purity independent of the extraneous materials. Therefore, any extraneous material present in the drug substance or active ingredient must be considered an impurity even if it is totally inert or has superior pharmacologic properties, so that an appropriate evaluation of its content in the drug product can be made. The control of low-level impurities is of great importance when a drug is taken in large quantities. Impurities can also affect the purity of API or can be harmful to patients.

CHARACTERIZATION OF IMPURITIES

The degradation samples were characterized by following methods

- UV spectroscopy
- INFRARED spectroscopy
- TLC
- HPLC

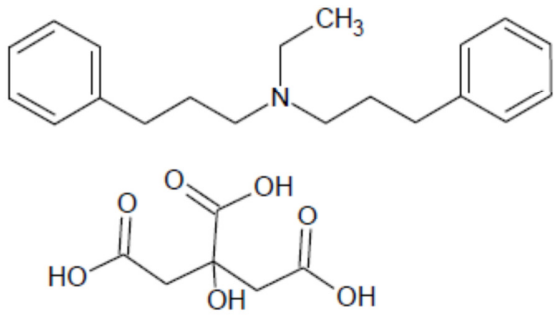
DRUG PROFILE

Alverine citrate is an anti cholinergic drug. Chemically it is an N-ethyl -3- phenyl-N-(3-phenyl propyl)-1-propanamine. It is an official drug of B.P(B.P. 2009)

EMPERICAL FORMULA: $C_{2O}H_{27}N$

MOLECULAR WEIGHT : 497.31

STRUCTURE



CATEGORY **:** Anti spasmodic

CAS number **:** 150-59-4

IUPAC NAME **:** Ethyl bis(3-phenyl propyl)amine

DESCRIPTION: White to pale yellow fine powder

SOLUBILITY: Slightly soluble in water and in methylene chloride, sparingly soluble in ethanol.

MELTING POINT: 104° C

PHARMACODYNAMICS

Alverine is a smooth muscle relaxant. Smooth muscle is a type of muscle that is not under voluntary control; it is the muscles present in places such as the gut and uterus. Alverine act directly through on the muscle in the gut, causing it to relax. This prevents the muscle spasms which occur in the gut in conditions such as irritable bowel syndrome and diverticular disease. Diverticular disease is a condition in which small pouches form in the gut lining. These pouches can trap particles of food and become inflamed and painful. It is also used to treat painful menstruation, which is caused by muscle spasm in the uterus. (Dysmenorrheal agent)

INDICATIONS

Alverine citrate is used to relieve cramps or spasms of the stomach and intestines. It is also useful in treating irritable bowel syndrome (IBS) and similar conditions. It can also be used to relieve period of pain. ALC is also under investigation to increase the cytotoxic effects of the proteasome inhibitor MG132on breast cancer cells.

METABOLISM

Rapidly converted to its primary metabolite, which is then further converted to two secondary metabolite

HALF LIFE

The plasma half -life averages 0.8 hours for Alverine and 5.7 hours for the active primary metabolite.

SIDE EFFECTS

Headache, Dizziness, itching, rash, jaundice.

TOXICITY:

Can produce hypotension and atropine –like toxic effects

DOSAGE:

60mg and 120mg of capsule

REVIEW OF LITERATURE

Alverine citrate is an official drug in British Pharmacopeia. Review of literature indicate that sophisticated analytical method like H & mass for estimating Alverine in biological fluids, and simple Spectrophotometric methods have been reported.

- **Niraimathi.V *et al.,* (2013)** Validated method development for the quantification of Alverine citrate by spectrophotometry, IR Spectrophotometry RP-HPLC in pharmaceutical dosage Form.

- **JU,Wwang *et al .,(*2012)**demonstrated the Dyclonine and ALC enhance cytotoxic effects of Proteasoma inhibitorMG132 on breast cancer cells

- **Rahul.C,*et al.,*(2011)** to developed liquid chromatography/tandem mass spectrometry for the simultaneous determination of Alverine and its metabolite, mono hydroxyl Alverine in human plasma.

- **T.Wittman, *et al.,*(2010)** had analyzed the efficacy of the ALC/simethicone combination on abdominal pain/discomfort in irritable bowel syndrome-a randomized, double-blind, placebo-controlled study

- **Chinmoy Ghosh, *et al.,* (2010)**had developed a rapid and most sensitive liquid chromatography/tandem mass spectrometry method for simultaneous determination of Alverine and its major metabolite, para hydroxyl Alverine in human plasma.

- **Wittmann *et al., .,* (2009)** has reported that ALC/simethicone combination was significantly more effective than placebo in relieving abdominal/pain.

- **Noel.A.Gomes, Ardhoot laud, *et al.,*(2009)** had developed validated LC-MS/MS method for determination of Alverine and one of its hydroxyl metabolites in human plasma along with its application to a bio equivalence study.

- **M.Hayase**, ***et al.,(*2007)** studied evolving mechanisms of ALC on phasic smooth muscle.

- **John W. Dolan (2002)** reported a stability indicating assays.

- **Monika Bakshi *et al., (*2002)** developed a stability indicating assay methods.

- **Coelho AM, *et al .,(*2001)**had studied the rectal antinociceptive properties of ALC are linked to antagonism at the 5-HTA,receptorsub type

- **FDA Guidance for Industry (2000)** gave the information on Analytical procedures and method validation.

- **Abysique A *et al.,*(1999)**studied the effects of ALC on cat intestinal mechanoreceptor response to chemical and mechanical stimuli.

- G**.J.Tudor, *et al .,(*1999)**diagnosed a general practice study to compare ALC with mebeverine hydrochloride in the treatment of irritable bowel syndrome

AIM AND OBJECTIVE

A degradation product is defined as a chemical change in the drug molecule brought about over time and/or by action of, e.g., light, temperature, pH, or water or by reaction with an excipient and/or the immediate container/closure system.

Stress testing is conducted to provide data on forced decomposition products and decomposition mechanisms. The severe conditions that may be encountered during distribution can be covered by stress testing.

Aim of the study is to conduct **"FORCED DEGRADATION STUDIES OF ALVERINE CITRATE IN BULK AND FORMULATION BY UV, IR SPECTROPHOTOMETRY, TLC, AND RP-HPLC METHOD".**

The study comprises of the following

- To perform the Alkali hydrolysis, Oxidative degradation, and Thermal and Photolytic degradation.
- Quantification of degraded samples by UV and HPLC.
- Comparing the results of bulk and sample with standard.
- Identification of degraded samples by TLC.
- Identification of changes in functional group present in the degraded samples by IR.

MATERIALS AND METHODS

MATERIALS AND INSTRUMENTS USED

DRUG SAMPLE & STUDY PRODUCTS

Alverine citrate was obtained from Apex Laboratories Ltd, Chennai, India.

Test product:

Alverine citrate capsules were purchased from local market.

CHEMICALS AND SOLVENTS USED FOR DEGRADATION:

- Hydrochloric Acid – Merck, AR grade, Mumbai, India.
- Hydrogen Peroxide (30%W/V) – Merck, AR grade, Mumbai, India.
- Sodium Hydroxide – Merck, AR grade , Mumbai, India
- Chloroform – Merck, AR grade , Mumbai, India
- Ethanol – Merck, AR grade , Mumbai, India
- Methanol – Merck, HPLC grade, Mumbai, India
- Acetonitrile – Merck, HPLC grade, Mumbai, India
- Water – Double Distilled

INSTRUMENTS USED:

- Shimadzu 1650 UV Spectrophotometer – Double beam , UV Probe2.31
- ABB-IR , KBr Press – AB MB 3000
- Agilent HPLC – VWD detector, Chemstation
- Shimadzu electronic balance – AX 200

FORCED DEGRADATION

Forced degradation studies are used to identify reactions which may occur to degrade a processed product. Purposeful degradation studies of the drug substance include appropriate solution and solid-state stress conditions (e.g., acid/base hydrolysis, heat, humidity, oxidation, and light exposure, in accordance with ICH guidelines). Guidelines from the United States Pharmacopoeia (USP), ICH, and FDA provide a brief outline of drug substance conditions.

ANALYTICAL PROCEDURES

Degraded samples were analyzed using Spectrophotometric methods like UV, IR and chromatographic methods like TLC and HPLC. The assays of degraded samples were carried out by UV and HPLC and the changes in the functional group of drug were detected by IR spectroscopy. Degraded products were identified by TLC.

HYDROLYTIC DEGRADTION

Hydrolytic stress testing is performed to force the degradation of a drug substance to its primary degradation products by exposure to acidic and basic conditions over time. Functional groups likely to introduce acid/base hydrolysis are amides (lactams), esters (lactones), carbamates, imides, imines, alcohols (epimerization for chiral centres), and aryl amines. To initiate acid/base studies, a preliminary solubility screen of the drug substance is performed. Solubility of at least 1 mg/mL in 0.1N acidic and basic condition is recommended for the acid/base stress testing; however, concentrations less than 1 mg/mL can be used if solubility is an issue. In some cases, a co-solvent may be necessary to achieve the target concentration.

OXIDATIVE DEGRADATION

The increase in oxidation state of an atom through a chemical reaction is known as an oxidation. Oxidative studies are executed to force the degradation of drug substances to determine the primary oxidative degradation products. Oxidative degradation is a serious stability problem and can cause a major halt in pharmaceutical development. Stability Guidelines state that a high oxygen atmosphere should be evaluated in stability studies on solutions or suspensions of the bulk drug substance. Mostly Oxidative degradation is done by Hydrogen peroxide. The concentration of hydrogen peroxide varies from 1% to 30%.

PHOTOLYTIC DEGRADATION

The goal of the photo stability studies is to force the degradation of drug substances via UV and fluorescent conditions over time to determine the primary degradation products. UV and visible light are the most energetic electromagnetic radiation sources to which pharmaceutical drug substances and drug products are typically exposed. A molecule absorbs light when an absorption band exists that overlaps to some extent with the incident light energy and a valence electron in the relevant chromophore is raised to an excited state. Light stress conditions can also induce photo oxidation by free radical mechanisms. The sample was placed in sun light to produce photolytic degradation.

THERMAL DEGRADATION

The goal of thermal and thermal studies is to force the degradation of drug substances over time to determine the primary thermal degradation products. Thermal degradation is used to detect the ability of samples withstand in different temperature. Thermal degradation was induced by placing the sample in hot oven. The temperature range for thermal degradation is from 40ºC to 100ºC.

1. UV SPECTROSCOPY

Ultraviolet Spectroscopy is concerned with the study of absorption of UV radiation which ranges from 200 nm to 400 nm. Any molecule has 'n' or 'π' or 'σ' or a combination of these electrons. These bonding (σ and π) and nonbonding (n) electrons absorb the characteristic radiation and undergoes transition from ground state to excited state. By the characteristic absorption peaks, the natures of the electrons present and hence the molecule structure can be elucidated. UV spectroscopy works on Beer Lamberts law Principle. UV is mainly used in the determination of assay value of samples based on the absorption of samples. In degradation studies UV can show the variation in maximum wavelength of the degraded samples. Degraded cannot give the same maximum wavelength as standard. Comparing the standard spectrum with degraded sample spectrum can differentiate the degradation.

Intraday scheme of UV study for Bulk& Formulation (Table: 1)

DEGRADATION	EXPERIMENTAL CONDITIONS	STORAGE CONDITION	SAMPLING TIME
	Control Sample (No acid or base)	Room temperature	30,60,90mins
Hydrolysis	0.1N HCL	Room temperature	30,60,90mins
	Acid Control (no API)	Room temperature	30,60,90mins
Oxidative	30%H_2O_2	Room temperature	30,60,90mins
	30%H_2O_2(no API)	Room temperature	30,60,90mins

Interday scheme of UV study for Bulk& Formulation (Table: 2)

DEGRADATION	EXPERIMENTAL CONDITIONS	STORAGE CONDITION	SAMPLING TIME
	Control Sample (No acid or base)	Room temperature	1,3,5 days
Hydrolysis	0.1N HCL	Room temperature	1,3,5 days
	Acid Control (no API)	Room temperature	1,3,5 days
Oxidative	30%H_2O_2	Room temperature	1,3,5 days
	30%H_2O_2(no API)	Room temperature	1,3,5 days
Thermal	Heating chamber	50ºC	1,3,5 days
Photolysis	Powder form	Sun Light	1,3,5 days

Materials and Methods

All absorption spectrums were measured by Shimadzu UV-1650PC spectrophotometer with a scanning speed of 200nm/min and a band width of 2.0nm equipped with 1cm matched quartz cells.

Reagents used

0.1N Hydrochloric acid

30% Hydrogen peroxide

Distilled water

PREPARATION OF REAGENTS

Preparation of 0.1N Sodium Hydroxide:

4gms of sodium hydroxide pellets were weighed and dissolved in small amount of distilled water then made up the volume to 1000mL.

Preparation of 0.1N Hydrochloric acid

8.33mL of concentrated Hydrochloric acid was measured and diluted with distilled water to 1000mL.

Preparation of 30% Hydrogen peroxide

300 mL of Hydrogen peroxide was diluted with distilled water and the volume made up to 1000mL.

1.1. INTRADAY STUDY OF HYDROLYTIC DEGRADATION USING 0.1N HCl

Standard preparation

Alverine citrate was transferred to volumetric flask and dissolved in distilled water to achieve a concentration of 1mg/mL. The solution was kept at room temperature. An aliquot solution was diluted with distilled water to get a final concentration of 100µg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 258nm.

Bulk preparation (stress)

25mg of Alverine citrate was transferred to volumetric flask and dissolved in 0.1N Hydrochloric acid to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with distilled water to get a final concentration of 100µg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 258nm. The same procedure was repeated for 60mins, and 90mins time interval.

Sample preparation (stress)

25mg equivalent of Alverine citrate capsules were weighed and transferred to volumetric flask; dissolved in 0.1N Hydrochloric acid was added to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with distilled water to get a final concentration of 100µg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 258nm. The same procedure was repeated for 60mins, and 90mins time interval.

Blank preparation

25mL of 0.1N HCl solution was taken in a 25mL volumetric flask. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with distilled water to get a final concentration. This is used as a blank.

The procedure was repeated thrice. After the stipulated time, the absorption of the resulting solution showed maxima 258nm against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated. The obtained values were tabulated.

The percentage content of bulk was determined by following formula

$$\textbf{Percentage content} = \frac{\textbf{Sam Abs.} \times \textbf{Std. Wt} \times \textbf{Dil. factor} \times \textbf{Purity of Std.} \times \textbf{100}}{\textbf{Std. Abs.} \times \textbf{Sam. Wt} \times \textbf{100}}$$

The amount of present was determined by following formula

$$\textbf{Amount present} = \frac{\textbf{Sam Abs.} \times \textbf{Std. Wt} \times \textbf{Dil. factor} \times \textbf{Purity of Std.} \times \textbf{Avg. Wt of tablets}}{\textbf{Std. Abs.} \times \textbf{Sam. Wt} \times \textbf{100}}$$

Percentage content of Alverine citrate was determined by following formula

$$\textbf{Percentage content} = \frac{\textbf{Amount present}}{\textbf{Label claim}}$$

OXIDATIVE DEGRADATION USING 30% H_2O_2

Bulk preparation (stress)

25mg of Alverine citrate was transferred to volumetric flask and dissolved in 30% Hydrogen peroxide to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with distilled water to get a final concentration of 100µg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 258nm. The same procedure was repeated for 60mins, and 90mins time interval**.**

Sample preparation (stress)

25mg equivalent of Alverine citrate capsules were weighed and transferred to volumetric flask; dissolved in 30% Hydrogen peroxide to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 30mins, the solution was scanned in the UV region and the maximum absorbance was recorded at 258nm. The same procedure was repeated for 60mins, and 90mins time interval.

Blank preparation

50mL of 30% H_2O_2 solution was taken in a 25mL volumetric flask. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with distilled water to get a final concentration. This is used as a blank.

The procedure was repeated thrice. After the stipulated time, the absorption of the resulting solution showed maxima 258nm against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated. The obtained values were tabulated.

1.2. INTERDAY HYDROLYTIC FORCED DEGRADATION STUDY USING 0.1N HCl

Bulk preparation (stress)

25mg of Alverine citrate was transferred to volumetric flask and 0.1N Hydrochloric acid to achieve a concentration of 1mg/mL. The solution was kept at room temperature. Then the next day, an aliquot solution was diluted with distilled water to get a final concentration of 100µg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 258nm. The same procedure was repeated for 3rd and 5th day time interval. The obtained spectrum is compared with standard spectrum.

Sample preparation (stress)

25mg equivalent of Alverine citrate capsules were crushed weighed and transferred to volumetric flask dissolved0.1N Hydrochloric acid to achieve a concentration of 1mg/mL. The solution was kept at room temperature. The next day (1st day), an aliquot solution was diluted with distilled water to get a final concentration of 100µg/mL.. The solution was scanned in the UV region and the maximum absorbance was recorded at 258nm. The same procedure was repeated for 3rd and 5th day time interval.

Blank preparation

25mL of 0.1N HCl solution was taken in a 25mL volumetric flask. The solution was kept at room temperature. The next day, an aliquot solution was diluted with distilled water to get a final concentration. This procedure is repeated for 3rd and 5th day.

The procedure was repeated thrice. After the stipulated time, the absorption of the resulting solution showed maxima 258nm against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated.

OXIDATIVE DEGRADATION USING 30% H_2O_2

Bulk preparation (stress)

25mg of Alverine citrate was weighed and transferred to volumetric flask and dissolved in 30% Hydrogen peroxide to achieve a concentration of 1mg/mL. The solution was kept at room temperature. Then the next day, an aliquot solution was diluted with distilled water to get a final concentration of 100µg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 258nm. The same procedure was repeated for 3rd and 5th day time interval. The obtained spectrum is compared with standard spectrum.

Sample preparation (stress)

25mg equivalent of Alverine citrate capsules were crushed weighed and transferred to volumetric flask dissolved in 30% Hydrogen peroxide to achieve a concentration of 1mg/mL. The solution was kept at room temperature. The next day (1st day), an aliquot solution was diluted with distilled water to get a final concentration of 100µg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 258nm. The same procedure was repeated for 3rd and 5th day time interval.

Blank preparation

25mL of 30% Hydrogen peroxide solution was taken in a 25mL volumetric flask. The solution was kept at room temperature. The next day, an aliquot solution was diluted with distilled water to get a final concentration. This procedure is repeated for 3rd and 5th day.

The procedure was repeated thrice. After the stipulated time, the absorption of the resulting solution showed maxima 258nm against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated.

THERMAL DEGRADATION AT 50ºC

Bulk preparation (stress)

1g of Alverine citrate bulk was weighed and transferred to a petri dish. This petri dish was placed in a hot air over at the temperature of 50ºC. The next day 25mg Alverine citrate bulk was weighed from a petri dish and transferred to 25mL volumetric flask. It was dissolved in distilled water and the volume made up to25mL. An aliquot solution was diluted with distilled water to get a final concentration of 100µg/mL. The same procedure was repeated for 3rd and 5th day.

Sample preparation (stress)

1g of Alverine citrate capsules were weighed and transferred to a petri dish. This petri dish was placed in a hot air over at the temperature of 50ºC. The next day 25mg equivalent of Alverine citrate capsule was weighed from a petri dish and transferred to 25mL volumetric flask. It was dissolved in distilled water and the volume made up to 25mL. An aliquot solution was diluted with distilled water to get a final concentration of 100µg/mL. the same procedure was repeated for 3rd and 5th day.

Blank preparation

Distilled water was used as blank

PHOTOLYTIC DEGRADATION USING SUN LIGHT

Bulk preparation (stress)

1g of Alverine citrate bulk was weighed and transferred to a petri dish. This petri dish was placed in a sun light. The next day 25mg Alverine citrate bulk was weighed from a petri dish and transferred to 25mL volumetric flask. It was dissolved in distilled water and the volume made up to 25mL. An aliquot solution was diluted with distilled water to get a final concentration of 100µg/mL. The same procedure was repeated for 3rd and 5th day.

Sample preparation (stress)

1g of Alverine citrate capsules were weighed and transferred to a petri dish. This petri dish was placed in a sun light. The next day 25mg equivalent of Alverine citrate was taken from the petri dish and transferred to 25mL volumetric flask. It was dissolved in distilled water and the volume made up to 25mL. An aliquot solution was diluted with distilled water to get a final concentration of 100µg/mL. The same procedure was repeated for 3rd and 5th day.

Blank preparation

Distilled water was used as a blank.

2. INFRARED SPECTROCOPY

An invaluable tool in organic structure determination and verification involves the class of electromagnetic (EM) radiation with frequencies between 4000 and 400cm^{-1} (Wavenumbers). The category of EM radiation is termed infrared (IR) radiation, and its application to organic chemistry known as IR spectroscopy. Radiation in this region can be utilized in organic structure determination by making use of the fact that it is absorbed by interatomic bonds in organic compounds. Chemical bonds in different environments will absorb varying intensities and at varying frequencies. Stretching absorptions usually produce stronger peaks than bending, however the weaker bending absorptions can be useful in differentiating similar types of bonds (e.g. aromatic substitution). It is also important to note that *symmetrical vibrations do not cause absorption of IR radiation*. IR is mainly used in the detection of functional groups present in the compounds. Comparing the standard spectrum with sample we can identify the changes of functional groups in the degraded sample. It is only applicable for solid samples undergoing degradation.

Interday scheme of IR study for Bulk& Formulation (Table: 3)

Degradation Type	Material(solid)	Storage condition	Sampling Time
Normal	Bulk	Room temperature	1,3,5days
	Sample	Room temperature	1,3,5days
Photolysis	Bulk	Sunlight	1,3,5days
	Sample	Sunlight	1,3,5days
Thermal(Heating Chamber)	Bulk	50ºC	1,3,5days
	Sample	50ºC	1,3,5days

Apparatus

All spectral measurements were made on ABB-IR (model no.MB3000) with KBr press (model no. M15).

General Procedure

Alverine citrate capsules were weighed and transferred into a petri dish. The first one was kept at room temperature, the second done was kept at chamber at 50ºC, and the third one was kept at sunlight. This was referred as 0 day. The bulk drug was weighed and transferred into 3 different petri dishes. The same procedure was repeated for bulk drug.

Standard preparation:

The first day standard of the Alverine citrate was weighed and reground with dry KBr using agate mortar and pestle. The KBr discs were prepared by using KBr pellet press instrument. Then the % transmittance of the standard was measured. The same procedure was repeated for $3^{rd}$and 5^{rd}day. The % transmittance was recorded in similar way.

Bulk preparation (stress)

The next day, required bulk drug has been taken from petri dish. The required amount of bulk drug was reground with dry KBr using agate mortar and pestle. The discs were prepared by using KBr press instrument. Then the percentage transmittance of the bulk drug was measured. The spectrum obtained from the degraded sample was compared with standard spectrum. The same procedure was repeated for 3^{rd} and 5^{th} day. The percentage transmittance was recorded in similar way.

Sample preparation (stress)

The next day (1^{st}) day, required sample has been taken from petri dish. The required amount of sample was reground with dry KBr using agate mortar and pestle. The discs were prepared by using KBr press instrument. Then the percentage transmittance of the sample was measured. The same procedure was repeated for 3^{rd} and 5^{th} day. The percentage transmittance was recorded in similar way.

THERMAL-50ºC:

Bulk preparation (stress)

The next day, the bulk from chamber was removed and the required quantity has been taken from the petri dish. It was kept in the same place. The required amount bulk drug was reground with dry KBr agate mortar and pestle .The discs were prepared by using KBr press instrument. Then the percentage transmittance of the bulk drug was measured. The same procedure was repeated for 3rd and 5th day. The percentage transmittance was recorded in similar way.

Sample preparation (stress)

Similar bulk procedure was followed.

SUNLIGHT:

Bulk preparation (stress)

The next day (1st) day, the required quantity has been taken from the petri dish. It was kept in the same place .The required amount of bulk was reground with dry KBr to using agate mortar and pestle. The discs were prepared by using KBr press instrument. Then the percentage transmittance of the bulk was measured .Compared the spectrum with standard spectrum. The same procedure was repeated foe 3rd and 5th day. The % transmittance was recorded in similar way.

Sample preparation (stress)

Similar bulk procedure was followed.

3. RP-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY:

High Performance Liquid Chromatography:

High performance Liquid Chromatography (HPLC) is a form of liquid chromatography to separate compounds that are dissolved in solution. This technique is based on the modes of separation like adsorption, partition, including reverse phase partition, ion–exchange and gel permeation. HPLC instrument consists of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector; Compounds are separated by injecting a plug of the sample mixture onto the column. The different compounds in the mixture pass through the column at different rates due to differences in their partitioning behaviour between the mobile liquid phase and the stationary phase.

Intraday scheme of HPLC study for Bulk& Formulation (Table no: 4)

DEGRADATION	EXPERIMENTAL CONDITIONS	STORAGE CONDITION	SAMPLING TIME
	Control Sample (No acid or base)	Room temperature	30,60,90mins
Hydrolysis	0.1N HCL	Room temperature	30,60,90mins
	Acid Control (no API)	Room temperature	30,60,90mins
Oxidative	30%H_2O_2	Room temperature	30,60,90mins
	30%H_2O_2(no API)	Room temperature	30,60,90mins

Interday scheme of HPLC study for Bulk& Formulation (Table: 5)

DEGRADATION	EXPERIMENTAL CONDITIONS	STORAGE CONDITION	SAMPLING TIME
Re	Control Sample (No acid or base)	Room temperature	1,3,5 days
Hydrolysis	0.1N HCL	Room temperature	1,3,5 days
	Acid Control (no API)	Room temperature	1,3,5 days
Oxidative	30%H_2O_2	Room temperature	1,3,5 days
	30%H_2O_2(no API)	Room temperature	1,3,5 days
Thermal	Heating chamber	50ºC	1,3,5 days
Photolysis	Powder form	Sun Light	1,3,5 days

Reagents and Chemicals Used For HPLC

- Acetonitrile HPLC Grade.
- Methanol
- Distilled water

Preparation of mobile phase:

Mobile phase was prepared with 50 % of Acetonitrile and 50% of Sodium Lauryl Sulphate (1:1) respectively. The mobile phase was prepared freshly, filtered through a 0.45µgm membrane filter and sonicated before use.

Standard preparation:

Alverine was transferred to volumetric flask and dissolved in methanol to achieve a concentration of 1mgmL^{-1} .An aliquot solution was diluted with distilled water to get a final concentration of 100µg/mL.

Bulk preparation:

25mg of Alverine bulk was weighed, transferred to volumetric flask and dissolved in distilled water to achieve a concentration of 1mg/mL. An aliquot solution was diluted with water to get a final concentration of 100µg/mL.

Sample preparation:

25mg equivalent of Alverine capsules were weighed transferred to volumetric flask and dissolved in distilled water to achieve a concentration of 1mg/mL. An aliquot solution was diluted with water to get a final concentration of 100µg/mL.

Before the sample, bulk and standard solutions were filtered through a 0.45µgm membrane filter.

Chromatographic conditions:

Column : C 18 (Reversed Phase)

Stationary Phase: Silica

Elution type : Isocratic

Mobile phase : Acetonitrile: Sodium Lauryl Sulphate (1:1)

Detector : PDA detector

Flow rate : 1 mL/min.

Determination of Retention Time:

The mobile phase was injected first to determine the absence of any interference with the base line. The retention time was then determined by injecting 20 µL of the standard in the column and the retention time was determined using 220 nm as the detection wavelength. The retention time was 6.8mins for Alverine citrate.

Analysis of sample solution:

The sample solution was diluted to get the required concentration and used for the estimation of Alverine citrate. 20 µg/mL of the solution was injected into the column, retention time and peak area was determined.

Assay:

20 µg/mL of standard and sample solution were injected separately with the flow rate of 1mL/minute of the mobile phase containing Acetonitrile: Sodium Lauryl Sulphate (1:1) proportion. The amount of Alverine citrate was calculated from the obtained chromatogram.

$$\mathbf{Amountpresent} = \frac{\mathbf{Sam.Area} \times \mathbf{Std.Wt} \times \mathbf{Dil.factor} \times \mathbf{Avg.Wt} \times \mathbf{PurityofStd}}{\mathbf{Std.Area} \times \mathbf{Sam.Wt} \times \mathbf{10}}$$

Percentage content of Alverine citrate

$$\mathbf{Percentage\ content} = \frac{\mathbf{Amount\ present}}{\mathbf{Label\ claim}}$$

3.1 INTRADAY STUDY BY RP-HPLC OF BULK AND FORMULATION

Hydrolytic degradation using 0.1N HCl

Bulk preparation (stress)

25mg of Alverine citrate was weighed and transferred to a 25mL volumetric flask. It was dissolved in methanol and the volume made up to 25mL using 0.1N HCl to achieve a concentration of 1mg/mL. After 90mins, an aliquot solution was diluted with mobile phase to get a concentration of 100µg/mL. The retention time and peak area were determined by recording the chromatograms.

Sample preparation (stress)

25mgequivalent of Alverine citrate capsules were weighed and transferred to a 25mL volumetric flask. It was dissolved in methanol and the volume made up to 25mL using0.1N HCl to achieve a concentration of 1mg/mL. After 90mins, an aliquot solution was diluted with mobile phase to get a concentration of 100µg/mL. The retention time and peak area were determined by recording the chromatograms.

Blank preparation

25mL of 0.1N HCl was taken in a 25mL volumetric flask. After 90mins, an aliquot solution was diluted with distilled water to get a final concentration. The blank solution was injected in to the column.

OXIDATIVE DEGRADATION USING 30% H_2O_2

Bulk preparation (stress)

25mg of Alverine citrate was weighed and transferred to a 25mL volumetric flask. It was dissolved in methanol and the volume made up to 25mL using 30% H_2O_2 to achieve a concentration of 1mg/mL. After 90mins, an aliquot solution was diluted with mobile phase to get a concentration of 100µg/mL. The retention time and peak area were determined by recording the chromatograms.

Sample preparation (stress)

25mg equivalent of Alverine citrate capsules were weighed and transferred to a 25mL volumetric flask. It was dissolved in methanol and the volume made up to 25mL using 30% H_2O_2 to achieve a concentration of 1mg/mL. After 90mins, an aliquot solution was diluted with mobile phase to get a concentration of 100µg/mL. The retention time and peak area were determined by recording the chromatograms.

Blank preparation

25mL of 30% H_2O_2 was taken in a 25mL volumetric flask. After 90mins, an aliquot amount of solution was used to get a final concentration. The blank solution was injected in to the column.

3.1 INTERDAY STUDY BY RP-HPLC OF BULK AND FORMULATION

Hydrolytic degradation using 0.1N HCl

Bulk preparation (stress)

25mg of Alverine citrate was weighed and transferred to a 25mL volumetric flask. It was dissolved in methanol and the volume made up to 25mL using 0.1N HCl to achieve a concentration of 1mg/mL. The third day, an aliquot solution was diluted with mobile phase to get a concentration of 100µg/mL. The retention time and peak area were determined by recording the chromatograms.

Sample preparation (stress)

25mg equivalent of Alverine citrate capsules were weighed and transferred to a 25mL volumetric flask. It was dissolved in methanol and the volume made up to 25mL using0.1N HCl to achieve a concentration of 1mg/mL. The third day, an aliquot solution was diluted with mobile phase to get a concentration of 100µg/mL. The retention time and peak area were determined by recording the chromatograms.

Blank preparation

25mL of 0.1N HCl was taken in a 25mL volumetric flask. The third day, an aliquot solution was diluted with distilled water to get a final concentration. The blank solution was injected in to the column.

OXIDATIVE DEGRADATION USING 30% H_2O_2

Bulk preparation (stress)

25mg of Alverine citrate was weighed and transferred to a 25mL volumetric flask. It was dissolved in methanol and the volume made up to 25mL using 30% H_2O_2 to achieve a concentration of 1mg/mL. The third day, an aliquot solution was diluted with mobile phase to get a concentration of 100µg/mL. The retention time and peak area were determined by recording the chromatograms.

Sample preparation (stress)

25mg equivalent of Alverine citrate capsules were weighed and transferred to a 25mL volumetric flask. It was dissolved in methanol and the volume made up to 25mL using 30% H_2O_2 to achieve a concentration of 1mg/mL. The third day, an aliquot solution was diluted with mobile phase to get a concentration of 100µg/mL. The retention time and peak area were determined by recording the chromatograms.

Blank preparation

25mL of 30% H_2O_2 was taken in a 25mL volumetric flask. The third day, an aliquot solution was diluted with distilled water to get a final concentration. The blank solution was injected in to the column.

THERMAL DEGRADATION AT 50ºC

Bulk preparation (stress)

1g of Alverine citrate bulk was weighed and transferred to a Petri dish. This Petri dish was placed in a hot air over at the temperature of 50ºC. The third day, 25mg Alverine citrate bulk was weighed from a Petri dish and transferred to 25mL volumetric flask. It was dissolved in mobile phase and the volume made up to 25mL. An aliquot solution was diluted with mobile phase to get a final concentration of 100µg/mL.

Sample preparation (stress)

1g of Alverine citrate capsules were weighed and transferred to a Petri dish. This Petri dish was placed in a hot air over at the temperature of 50ºC. The third day, 25mg equivalent of Alverine citrate tablet was weighed from a Petri dish and transferred to 25mL volumetric flask. It was dissolved in ethanol and the volume made up to 25mL. An aliquot solution was diluted with mobile phase to get a final concentration of 100µg/mL.

PHOTOLYTIC DEGRADATION USING SUN LIGHT

Bulk preparation (stress)

1g of Alverine citrate bulk was weighed and transferred to a petri dish. This petri dish was placed in a sun light. The 3rd day 25mg Alverine citrate bulk was weighed from a petri dish and transferred to 25mL volumetric flask. It was dissolved in ethanol and the volume made up to 25mL. An aliquot solution was diluted with mobile phase to get a final concentration of 100µg/mL.

Sample preparation (stress)

1g of Alverine citrate capsules were weighed and transferred to a petri dish. This petri dish was placed in a sun light. The 3rd day 25mg equivalent of Alverine citrate was taken from the petri dish and transferred to 25mL volumetric flask. It was dissolved in ethanol and the volume made up to 25mL. An aliquot solution was diluted with mobile phase to get a final concentration of 100µg/mL.

4. THIN LAYER CHROMATOGRAPHY

Thin Layer Chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures. TLC is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose. This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved. Thin layer chromatography can be used to monitor the progress of a reaction, identify the compounds present in a given mixture, and determine the purity of a substance. In degradation studies it is used to

- To identify the degradation(whether the degradation occurred or not)
- To detect impurities (like no. of impurities formed)

Presences of impurities were detected by number of spots and intensity of spots after the detection. Various mobile phases are given below tried for Alverine citrate.

- Hexane: Ethyl acetate (6:4)
- Chloroform: Toluene: Methanol (6:2:2)
- Chloroform: Methanol: Water (6:2:2)
- Chloroform: Methanol (5:5)
- Chloroform: Ethanol (9:1)

Mobile phase containing Chloroform and Methanol (9:1) were chosen for the study as it gave better resolution. Detection was carried out at by using iodine chamber.

Rf values were calculated by following formula

$$Rf = \frac{Distance\ travelled\ by\ solute}{Distance\ travelled\ by\ solvent}$$

Mobile phase preparation

Mobile phase was prepared by using chloroform and methanol in the ration of 9:1. Then the mobile phase was allowed for saturation. Mobile phase should be prepared freshly.

Interday scheme of study of Bulk & Formulation (Table: 6)

DEGRADATION	EXPERIMENTAL CONDITIONS	STORAGE CONDITION	SAMPLING TIME
	Control Sample (No acid or base)	Room temperature	90mins
Hydrolysis	0.1N HCl	Room temperature	90mins
	Acid Control (no API)	Room temperature	90mins
Oxidative	30%H_2O_2	Room temperature	90mins
	30%H_2O_2(no API)	Room temperature	90mins

Interday scheme of study of Bulk & Formulation (Table: 7)

DEGRADATION	EXPERIMENTAL CONDITIONS	STORAGE CONDITION	SAMPLING TIME
	Control Sample (No acid or base)	Room temperature	3rd day
Hydrolysis	0.1N HCl	Room temperature	3rd day
	Acid Control (no API)	Room temperature	3rd day
Oxidative	30%H_2O_2	Room temperature	3rd day
	30%H_2O_2(no API)	Room temperature	3rd day
Thermal	Heating chamber	50ºC	3rd day
Photolysis	Powder form	Sunlight	3rd day

4.1. INTRADAY HYDROLYTIC DEGRADATION USING 0.1N HCl

Preparation of standard

Small amount of Alverine citrate was dissolved in methanol and used as a standard. Standard should be prepared freshly.

Detecting agent: Iodine chamber

Bulk preparation (stress)

25mg of Alverine citrate was transferred to volumetric flask and dissolved in 0.1N Hydrochloric acid to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 90mins, the sample was spotted on the TLC plate using micropipette. This was marked as P. Then standard was spotted and denoted as D. Then the TLC was placed in the mobile phase containing TLC chamber for elution. After sufficient elution TLC plate was removed from the TLC chamber and placed in an iodine chamber for detection. Rf value was calculated.

Sample preparation (stress)

25mg equivalent of Alverine citrate capsules were weighed and transferred to volumetric flask; dissolved in 0.1N Hydrochloric acid was added to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 90mins, the sample was spotted on the TLC plate using micropipette. This was marked as P. Then standard was spotted and denoted as D. Then the TLC was placed in the TLC chamber containing mobile phase for elution. After sufficient elution TLC plate was removed from the TLC chamber and placed in an iodine chamber for detection.

OXIDATIVE DEGRADATION USING 30% H_2O_2

Bulk preparation (stress)

25mg of Alverine citrate was weighed and transferred to volumetric flask and dissolved in 30% Hydrogen peroxide to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 90mins, the sample was spotted on the TLC plate using micropipette. This was marked as P. Then standard was spotted and denoted as D. Then the TLC was placed in the TLC chamber containing mobile phase for elution. After sufficient elution TLC plate was removed from the TLC chamber and placed in an iodine chamber for detection.

Sample preparation (stress)

25mg equivalent of Alverine citrate capsules were weighed and transferred to volumetric flask dissolved in 30%Hydrogen peroxide to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 90mins, the sample was spotted on the TLC plate using micropipette. This was marked as P. Then standard was spotted and denoted as D. Then the TLC was placed in the TLC chamber containing mobile phase for elution. After sufficient elution TLC plate was removed from the TLC chamber and placed in an iodine chamber for detection.

4.2. INTERDAY FORCED DEGRADATION STUDY USING 0.1N HCl and 30% H_2O_2

Same as the intraday procedure was adopted for the interday degradation. After three days the bulk and sample were collected.

THERMAL DEGRADATION AT 50ºC

Bulk preparation (stress)

1g of Alverine citrate bulk was weighed and transferred to a petri dish. This petri dish was placed in a hot air over at the temperature of 50ºC. The third day, the sample was spotted on the TLC plate using micropipette. This was marked as P. Then standard was spotted and denoted as D. Then the TLC was placed in the TLC chamber containing mobile phase for elution. After sufficient elution TLC plate was removed from the TLC chamber and placed in an iodine chamber for detection.

Sample preparation (stress)

1g of Alverine citrate capsules were weighed and transferred to a petri dish. This petri dish was placed in a hot air over at the temperature of 50ºC. The third day, the sample was spotted on the TLC plate using micropipette. This was marked as P. Then standard was spotted and denoted as D. Then the TLC was placed in the TLC chamber containing mobile phase for elution. After sufficient elution TLC plate was removed from the TLC chamber and placed in an iodine chamber for detection.

PHOTOLYTIC DEGRADATION USING SUN LIGHT

Bulk preparation stress

1g of Alverine citrate bulk was weighed and transferred to a petri dish. This petri dish was placed in a sun light. The third day, the sample was spotted on the TLC plate using micropipette. This was marked as P. Then standard was spotted and denoted as D. Then the TLC was placed in the TLC chamber containing mobile phase for elution. After sufficient elution TLC plate was removed from the TLC chamber and placed in an iodine chamber for detection. .

Sample preparation

1g of Alverine citrate capsules were weighed and transferred to a petri dish. This petri dish was placed in a sun light. The third day, the sample was spotted on the TLC plate using micropipette. This was marked as P. Then standard was spotted and denoted as D. Then the TLC was placed in the TLC chamber containing mobile phase for elution. After sufficient elution TLC plate was removed from the TLC chamber and placed in an iodine chamber for detection.

RESULTS AND DISCUSSION

HYDROLYTIC DEGRADATION STUDY USING 0.1 N HYDROCHLORIC ACID

Hydrolytic degradation was performed as per the procedure in materials and methods. The assay value of standard and sample were evaluated by spectrophotometry. The respective UV- spectrum and the values are given in Fig.1-4, Table-8, 9.

Fig. 1 Overlay Spectrum of Alverine Citrate Bulk with Standard in 0.1N HCl

30mins **90mins**

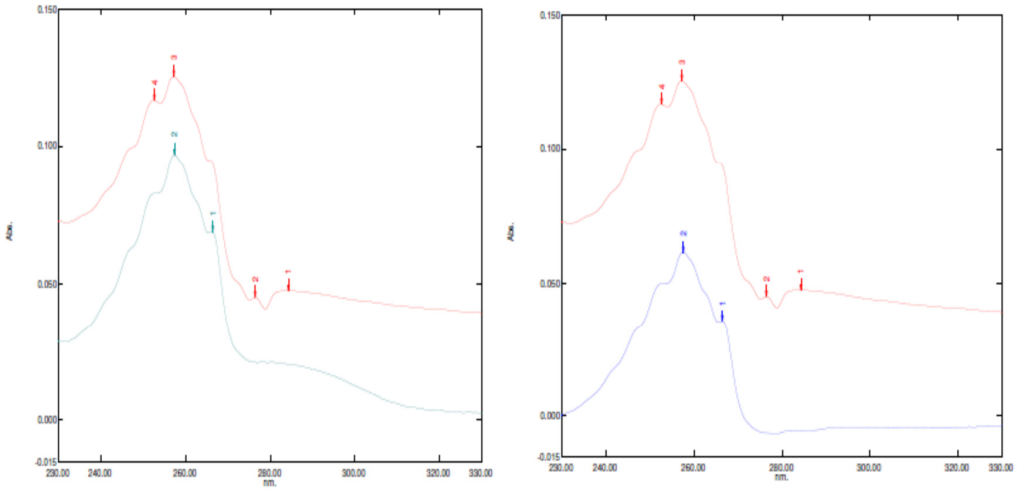


Fig. 2 Overlay Spectrum of Alverine Citrate Sample with Standard in 0.1N HCl

30mins **90mins**

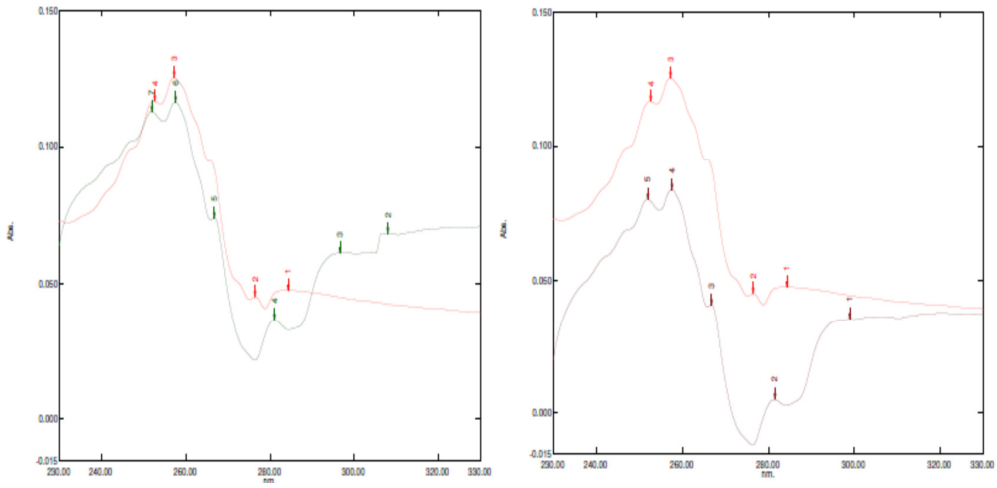


Fig. 3 Overlay Spectrum of Alverine Citrate Bulk with Standard in 0.1N HCl

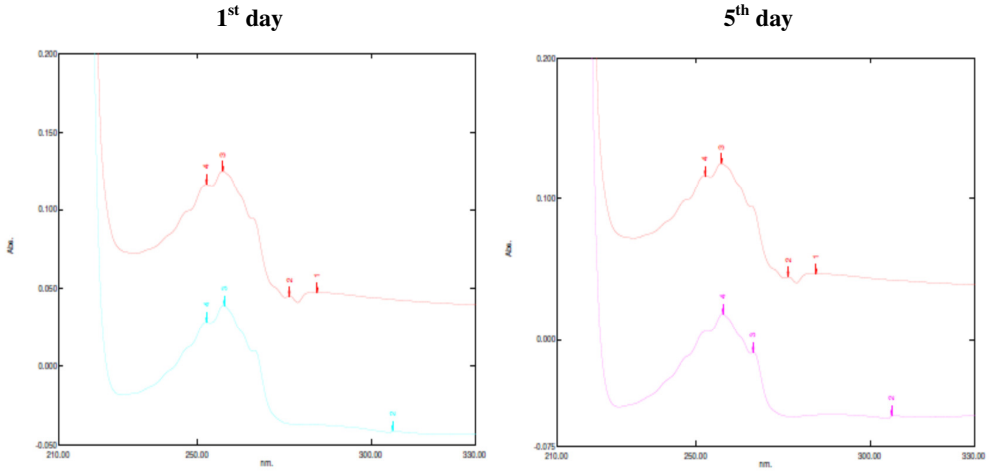


Fig. 4 Overlay Spectrum of Alverine Citrate Sample with Standard in 0.1N HCl

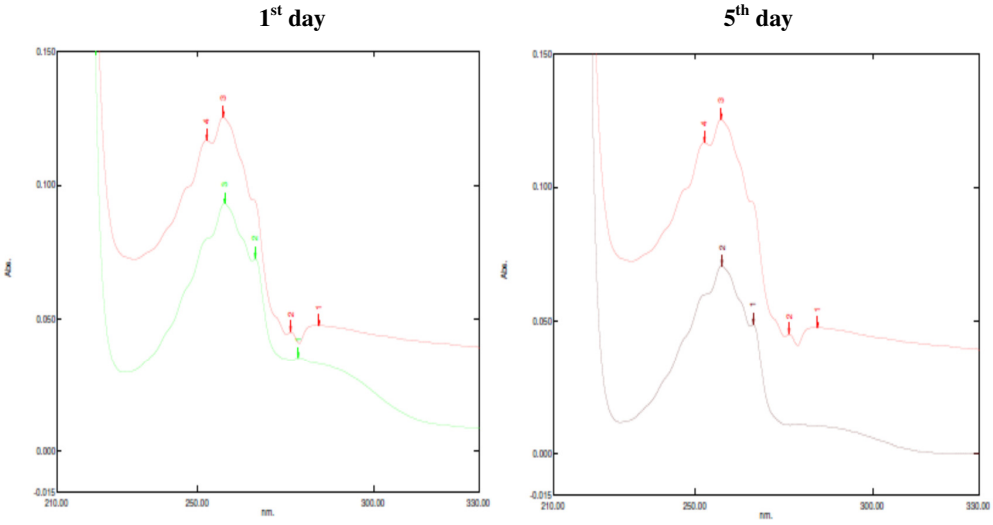


Table 8: Absorbance Values for Hydrolytic Degradation Using 0.1 N HCl

S.No.	Drug	Absorbance	Standard	Time
1	Bulk	0.097	0.125	30mins
2		0.101		
3		0.093		
1	Sample	0.116		
2		0.120		
3		0.122		
1	Bulk	0.072	0.125	60mins
2		0.080		
3		0.064		
1	Sample	0.097		
2		0.105		
3		0.089		
1	Bulk	0.061	0.125	90mins
2		0.066		
3		0.056		
1	Sample	0.084		
2		0.078		
3		0.090		

Table.8.1: Results obtained from hydrolytic degradation -0.1 N HCl

Stress condition (Acid Hydrolysis)	Time	Bulk Percentage content (%)	Sample percentage content (%)
0.1 N HCl	30mins	77.6	92.8
	60mins	57.6	77.6
	90mins	48.8	67.2

❖ Each value is the mean of three determinations.

1.2. INTERDAY STUDY:

Table 9: Absorbance Values For Hydrolytic Degradation Using - 0.1 N HCl

S.No.	Drug	Absorbance	Standard	Time
1	Bulk	0.039	0.126	1st day
2		0.045		
3		0.033		
1	Sample	0.093		
2		0.099		
3		0.087		
1	Bulk	0.072	0.126	3rd day
2		0.081		
3		0.063		
1	Sample	0.087		
2		0.098		
3		0.076		
1	Bulk	0.019	0.126	5th day
2		0.022		
3		0.016		
1	Sample	0.070		
2		0.080		
3		0.060		

Table.9.1: Results obtained from hydrolytic degradation - 0.1 N HCl

Stress condition (Alkali hydrolysis)	Time	Bulk Percentage content (%)	Sample percentage content (%)
0.1 N Hydrochloric acid	1st day	31.2	74.4
	3rd day	16.8	69.6
	5th day	15.1	56.0

❖ Each value is the mean of three determinations.

GRAPHICAL REPRESENTATION OF INTRADAYAND INTER DAY STUDY

Fig: 5 Assay Values of Bulk and Sample at Various Time Intervals

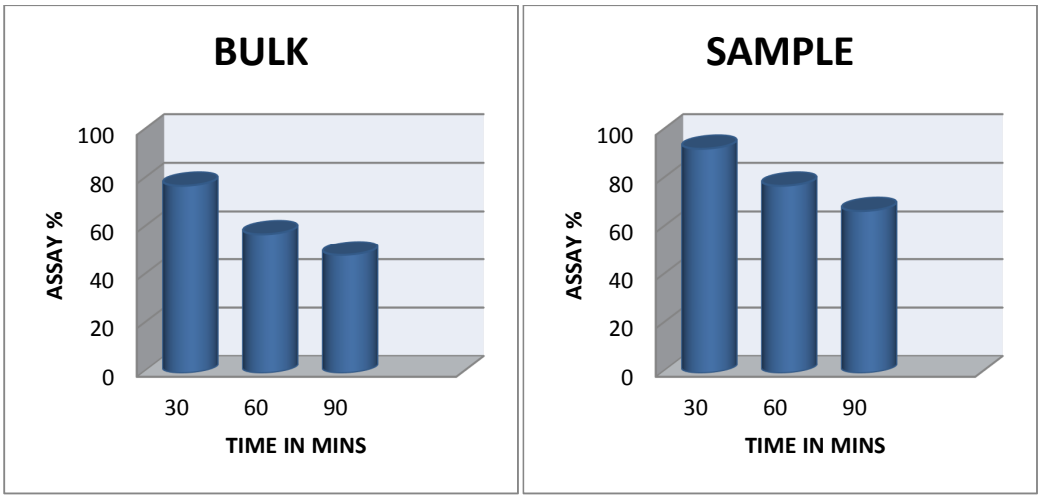
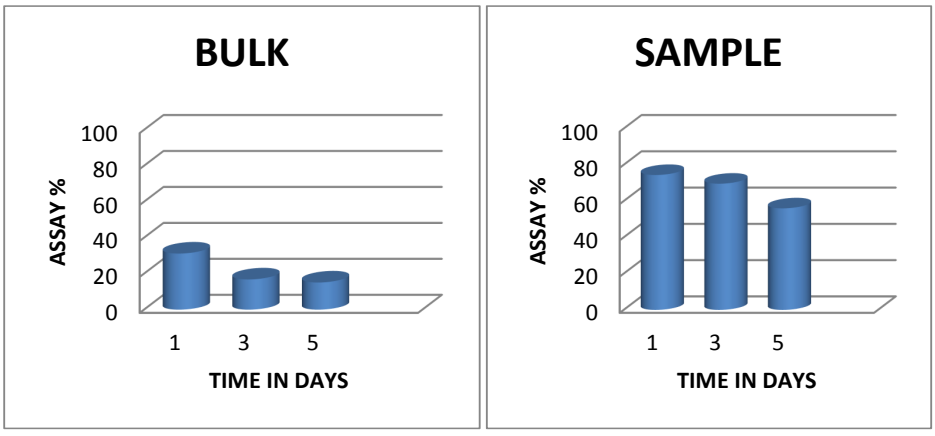


Fig: 6 Assay Values of Bulk and Sample at Various Time Intervals



Alverine citrate was found to be unstable under acidic condition. Table: 8 shows the results of intraday degradation and the amount of standard and sample remaining in the solution after certain time intervals. The assay values of standard and the sample were found to be 48.8% and 74.4% at the end of 90mins. The results of Interday degradation are given in Table: 9 The assay value of standard and sample was found to be 31.2% and 69.6% at the end of the first day acid hydrolysis. At the end of the 5^{th} day the assay values of standard and sample were found to be 15.2% and 56.0%.

OXIDATIVE DEGRADATION USING 30% H_2O_2

Oxidative degradation was performed as per the procedure in materials and methods .the assay value of standard and sample were evaluated by spectrophotometry. The respective UV- spectrum and the values are given in Fig.7-10, Table-10, 11.

Fig. 7 Overlay Spectrum of Alverine Citrate Bulk with Standard in 30% H_2O_2

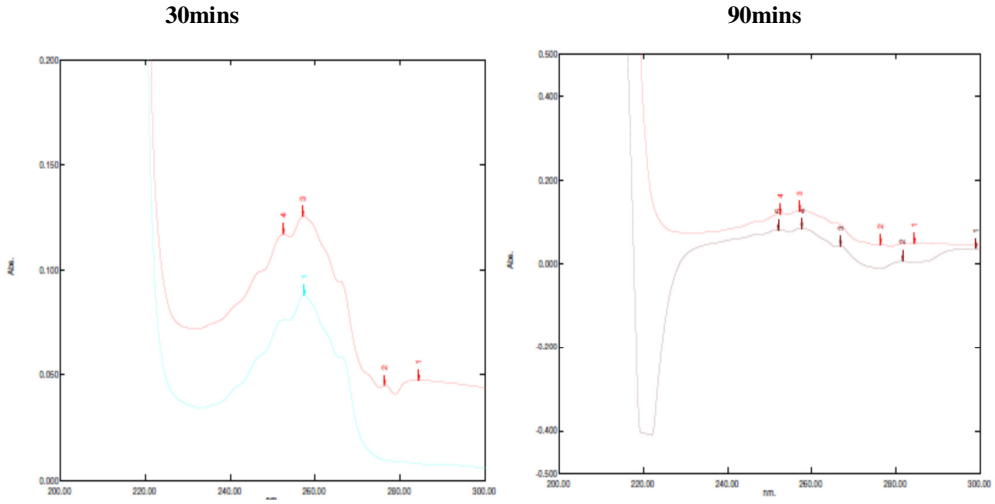


Fig. 8 Overlay Spectrum of Alverine Citrate Sample with Standard in 30% H_2O_2

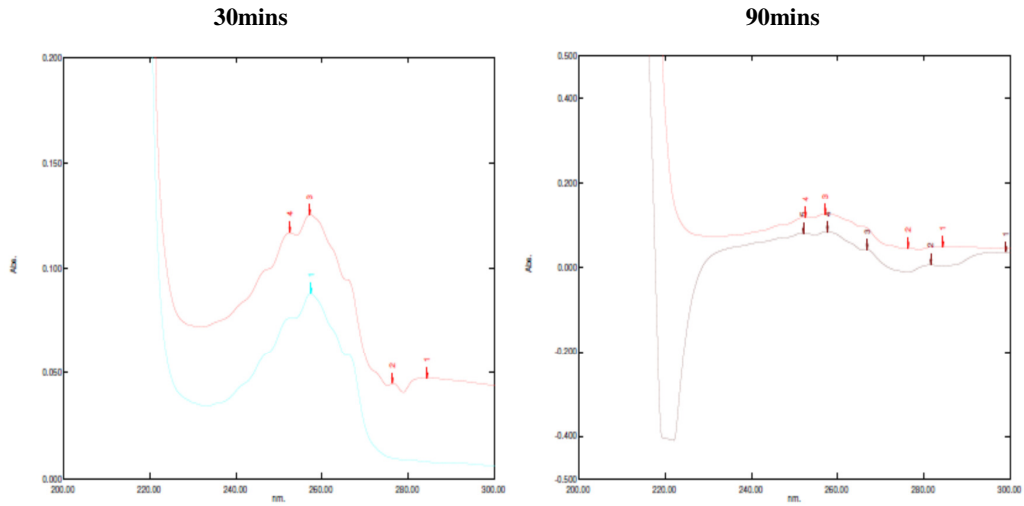


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Fig. 9 Overlay Spectrum of Alverine Citrate Bulk with Standard in 30% H_2O_2

1st day **5th day**

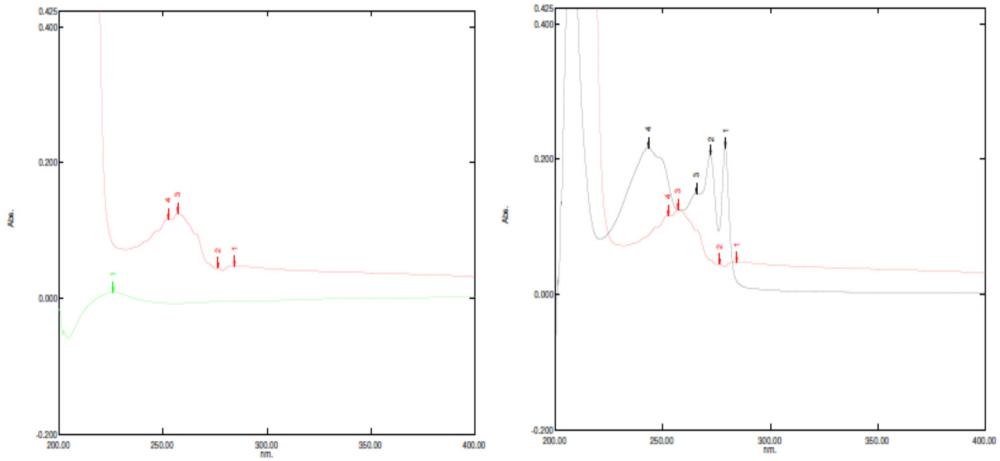


Fig.10 Overlay Spectrum of Alverine Citrate Sample with Standard in 30% H_2O_2

1st day **5th day**

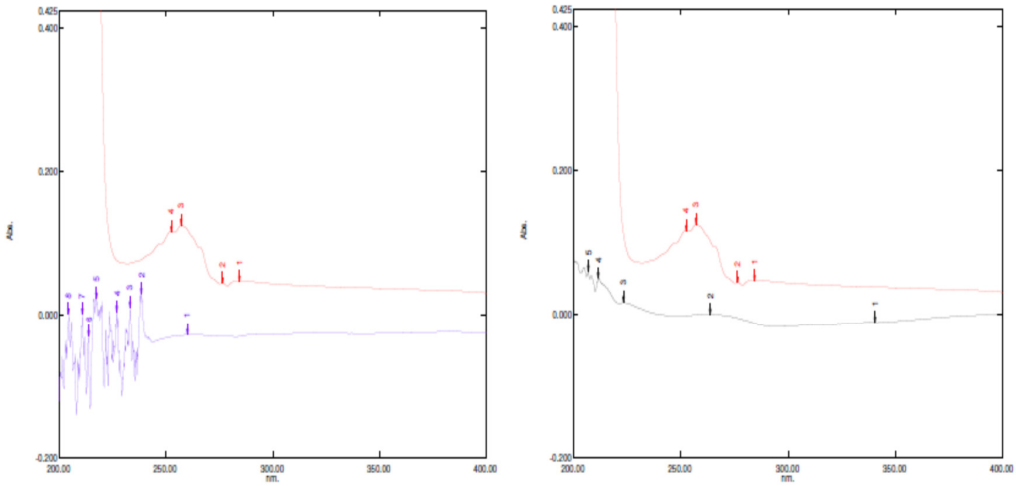


Table 10: Absorbance Values for Oxidative Degradation Using - 30% H_2O_2

S.No.	Drug	Absorbance	Standard	Time
1	Bulk	0.090	0.124	30mins
2		0.082		
3		0.098		
1	Sample	0.097		
2		0.105		
3		0.089		
1	Bulk	0.088	0.125	60mins
2		0.098		
3		0.078		
1	Sample	0.087		
2		0.081		
3		0.093		
1	Bulk	0.082	0.128	90mins
2		0.088		
3		0.076		
1	Sample	0.082		
2		0.072		
3		0.092		

Table.10.1: Results Obtained From Oxidative Degradation - 30% H_2O_2

Stress condition (Oxidation)	Time	Bulk Percentage content (%)	Sample percentage content (%)
30% H_2O_2	30mins	71.9	77.6
	60mins	70.3	69.6
	90mins	65.5	65.6

❖ Each value is the mean of three determinations.

Table 11: Absorbance Values for Oxidative Degradation Using - 30% H_2O_2

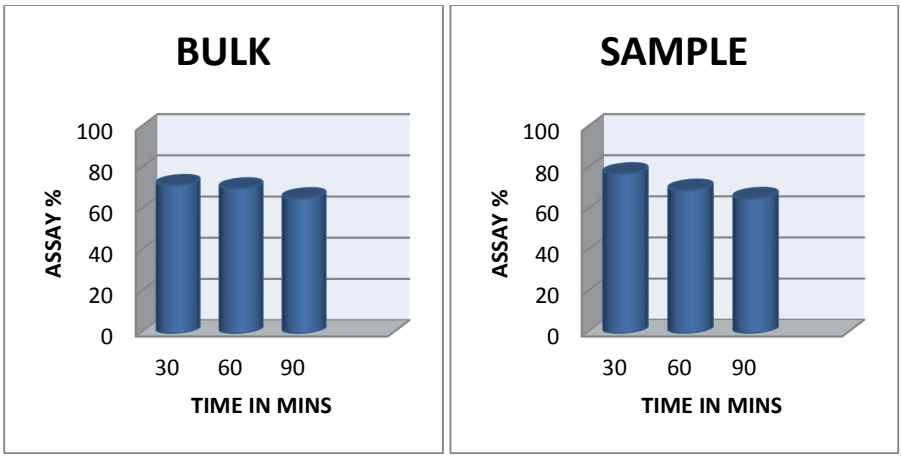
S.No.	Drug	Absorbance	Standard	Time
1	Bulk	0	0.125	1st day
2		0		
3		0		
1	Sample	0		
2		0		
3		0		

Table.11.1: Results Obtained From Oxidative Degradation - 30% H_2O_2

Stress condition (Oxidation)	Time	Bulk Percentage content (%)	Sample percentage content (%)
30% H_2O_2	1st day	0	0
	3rd day	0	0
	5th day	0	0

GRAPHICAL REPRESENTATION OF INTRADAY STUDY

Fig: 11 Assay Values of Bulk and Sample at Various Time Intervals



Alverine citrate was found to be unstable under oxidative stress condition. It was found that the stress induced standard and the test were found to degrade to 68.3% and 68.0% at the end of 90mins. The assay value of standard and sample was found to be 0% at the end of the first day of oxidative degradation.

THERMAL DEGRADATION

Thermal degradation was performed as per the procedure in materials and methods. The assay value of standard and sample were evaluated by UV-spectrophotometry. The respective UV- spectrum and the values are given in Fig.12-13, Table-12.

Fig. 12 Overlay Spectrum of Alverine Citrate Bulk with Standard

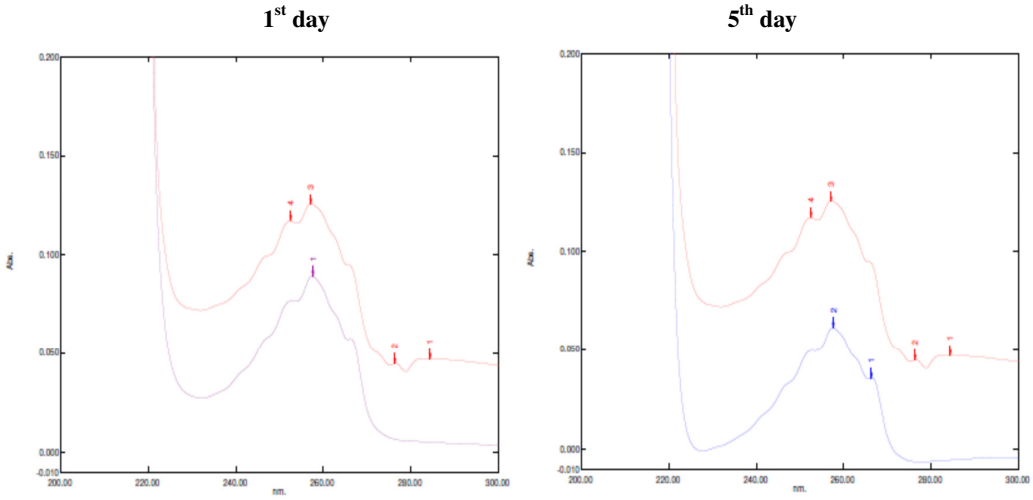


Fig. 13 Overlay Spectrum of Alverine Citrate Sample with Standard

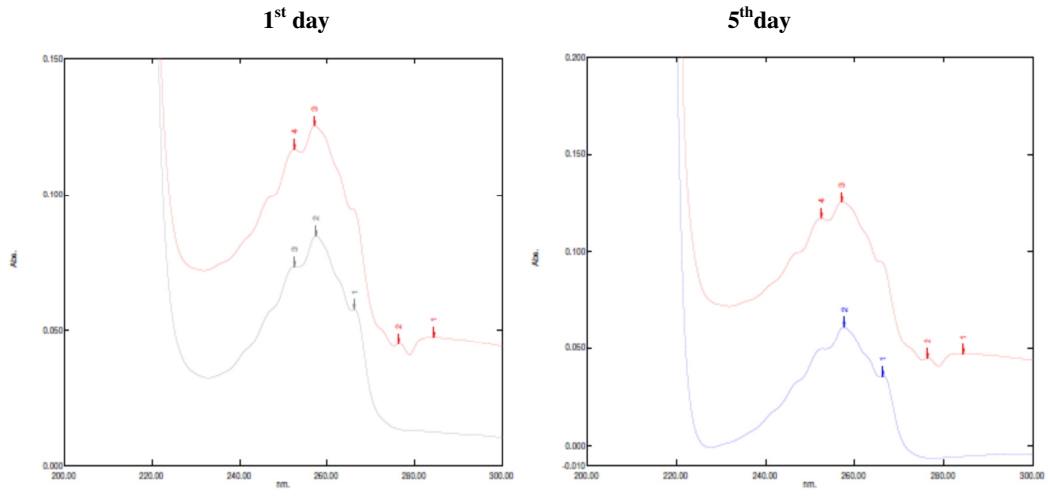


Table 12: Absorbance Values for Thermal at 50ºC

S.No.	Drug	Absorbance	Standard	Time
1	Bulk	0.085	0.126	1st day
2		0.090		
3		0.080		
1	Sample	0.089		
2		0.098		
3		0.081		
1	Bulk	0.065	0.124	3rd day
2		0.073		
3		0.058		
1	Sample	0.078		
2		0.071		
3		0.085		
1	Bulk	0.061	0.126	5th day
2		0.055		
3		0.067		
1	Sample	0.061		
2		0.067		
3		0.065		

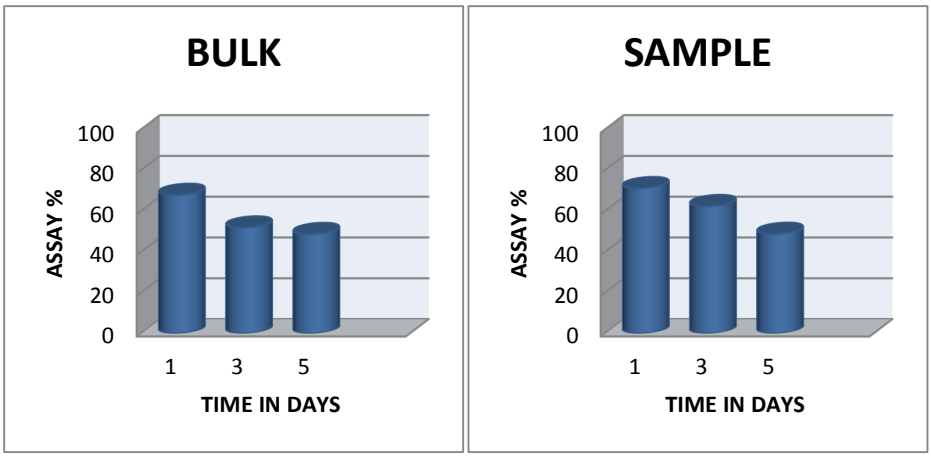
Table.12.1: Results Obtained from Thermal degradation - 50ºC

Stress Condition (Thermal)	Time	Bulk Percentage Content (%)	Sample Percentage Content (%)
50ºC	1st day	67.9	71.2
	3rd day	51.9	62.4
	5th day	48.7	48.8

- Each value is the mean of three determinations.

GRAPHICAL REPRESENTATION OF INTERDAY STUDY

Fig: 14 Assay Values of Bulk and Sample at Various Time Intervals



Extensive degradation was observed in both standard and sample. The assay values of standard and sample was found to be 48.8%. It indicates that Alverine citrate was vulnerable to heat.

PHOTOLYTIC DEGRADATION

Alverine Citrate bulk and sample were kept in sunlight. The Bulk and Sample powders were collected at different time intervals and the assay values were calculated by UV spectroscopy. The respective UV spectrum and the values are given in Fig.15-17, Table-13.

Fig.16 Overlay Spectrum of Alverine Citrate Bulk with Standard

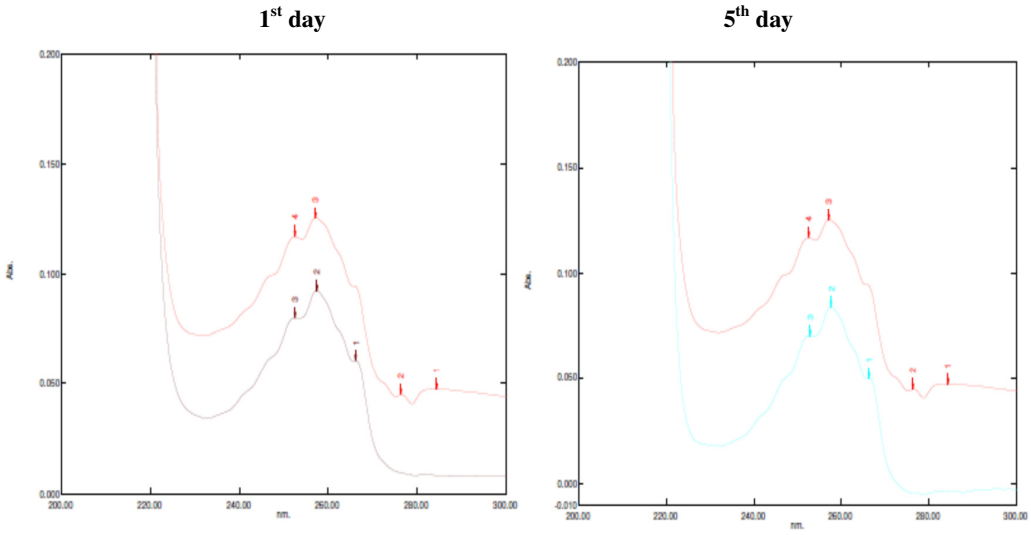


Fig.17 Overlay Spectrum of Alverine Citrate Sample with Standard

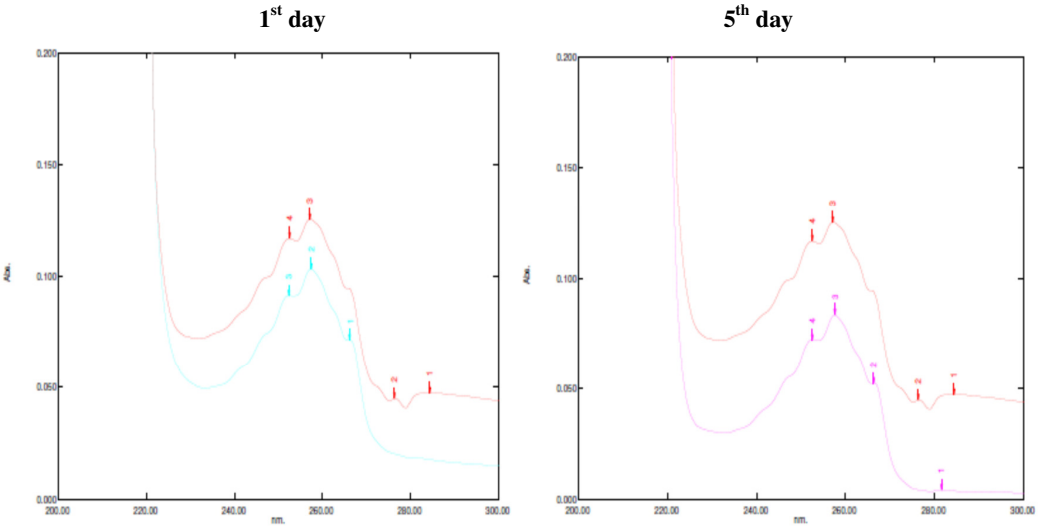


Table 13: Absorbance Values for Photolytic Degradation Using Sunlight

S.No.	Drug	Absorbance	Standard	Time
1	Bulk	0.093	0.128	1st day
2		0.100		
3		0.060		
1	Sample	0.103		
2		0.110		
3		0.096		
1	Bulk	0.090	0.125	3rd day
2		0.100		
3		0.080		
1	Sample	0.088		
2		0.099		
3		0.077		
1	Bulk	0.084	0.126	5th day
2		0.091		
3		0.077		
1	Sample	0.084		
2		0.088		
3		0.080		

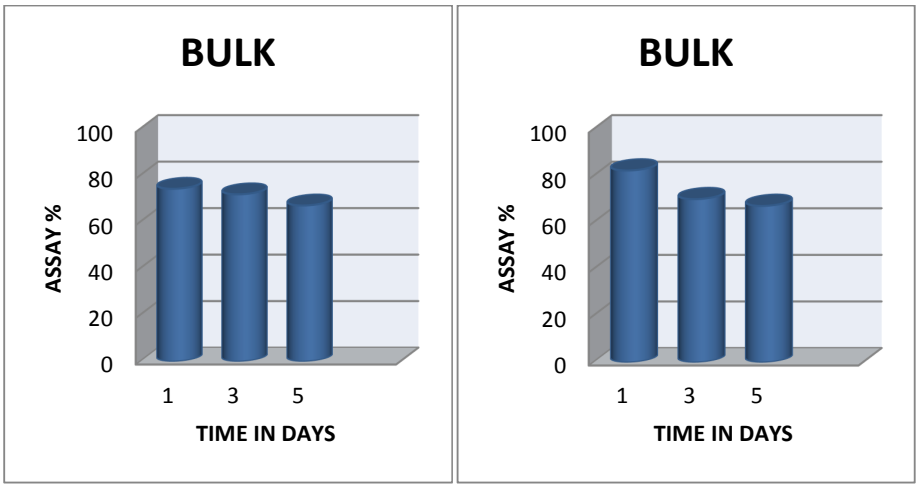
Table.13.1 Results Obtained After Photolytic Degradation –Sunlight

Stress Condition (Photolytic)	Time	Bulk Percentage Content (%)	Sample Percentage Content (%)
Sunlight	1st day	74.3	82.4
	3rd day	71.9	70.4
	5th day	67.1	67.2

- Each value is the mean of three determinations.

GRAPHICAL REPRESENTATION OF INTERDAY STUDY

Fig: 18 Assay Values of Bulk and Sample at Various Time Intervals



Alverine citrate sample and standard underwent photolytic degradation in presence of sunlight. The assay values of standard and sample were found to be 67.1% and 67.2% respectively. Therefore the formulations of Alverine where have to be protected from light exposure.

2) IR STUDY OF BULK&SAMPLE-UNDER STRESS &NORMAL CONDITION:

In interday Thermal and photolytic degradation the samples were kept in heating chamber and sunlight. The next day the samples were taken and ground with KBr. Then the KBr pellets were formed using pellet pressing technique. IR spectrum was taken for pellets. IR spectrum is given in Fig.19-26, Table-14.

Thermal Degradation at 50ºC

Fig: 19 Overlay IR Spectrum of Standard with Bulk in 1st Day

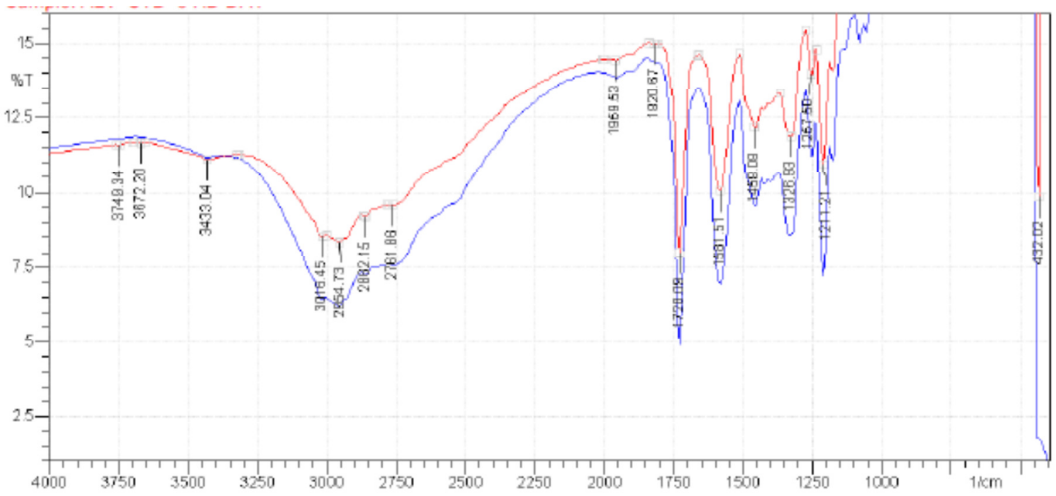


Fig: 20 Overlay IR Spectrum of Standard with Bulk 5th Day

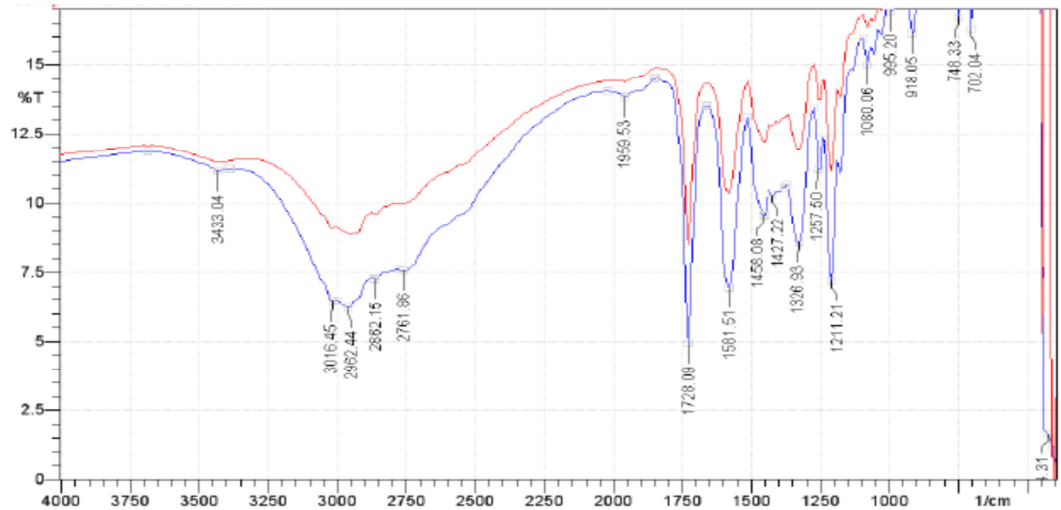


Fig: 21 Overlay IR Spectrum of Standard with Sample in 1st Day

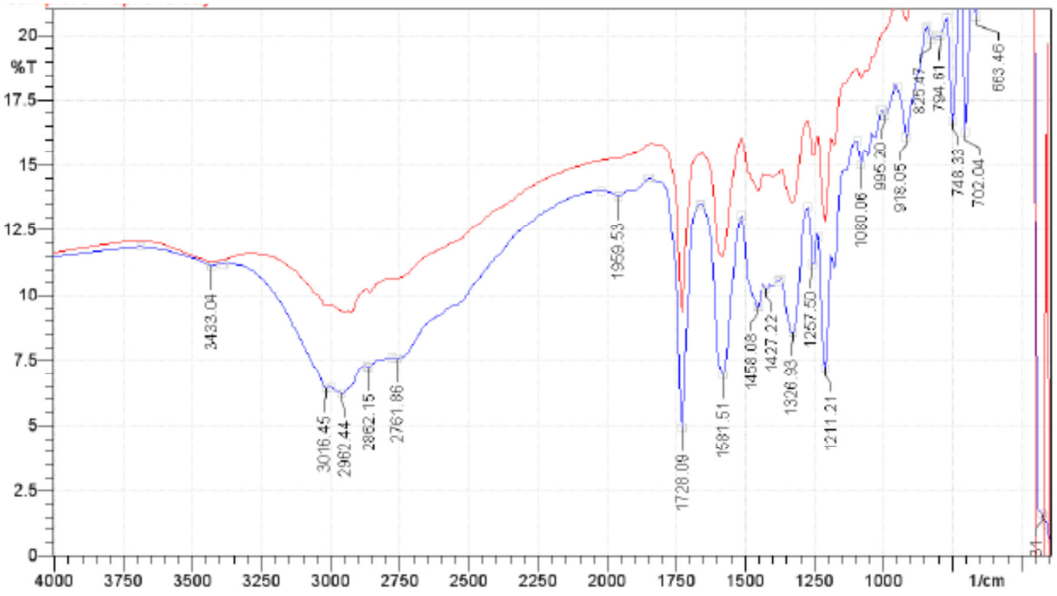
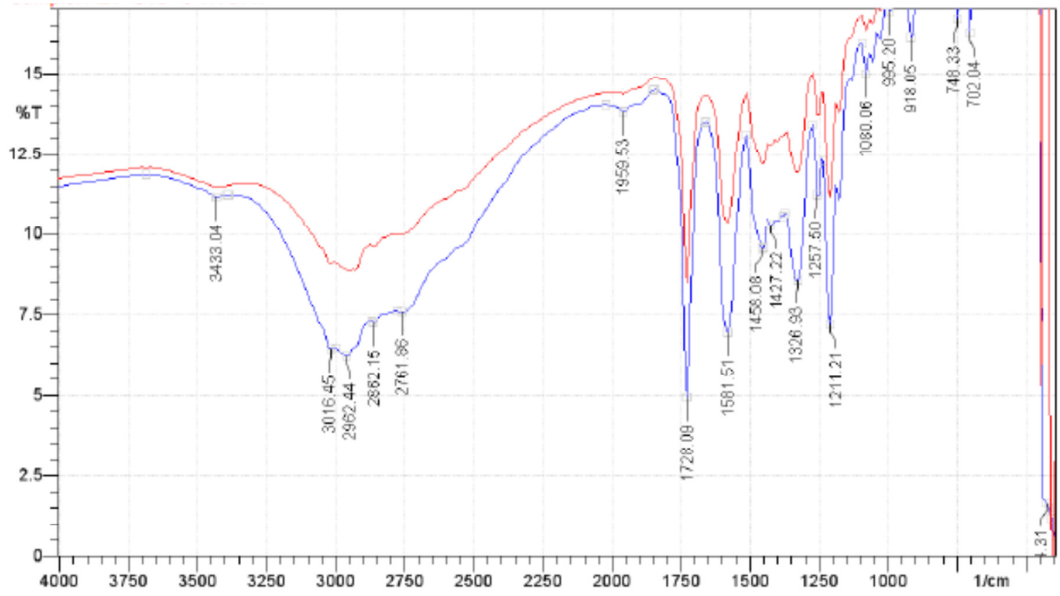


Fig: 22 Overlay IR Spectrum of Standard with Sample in 5th Day



Photolytic Degradation at 50ºC

Fig: 23 Overlay IR Spectrum of Standard with Bulk in 1st Day

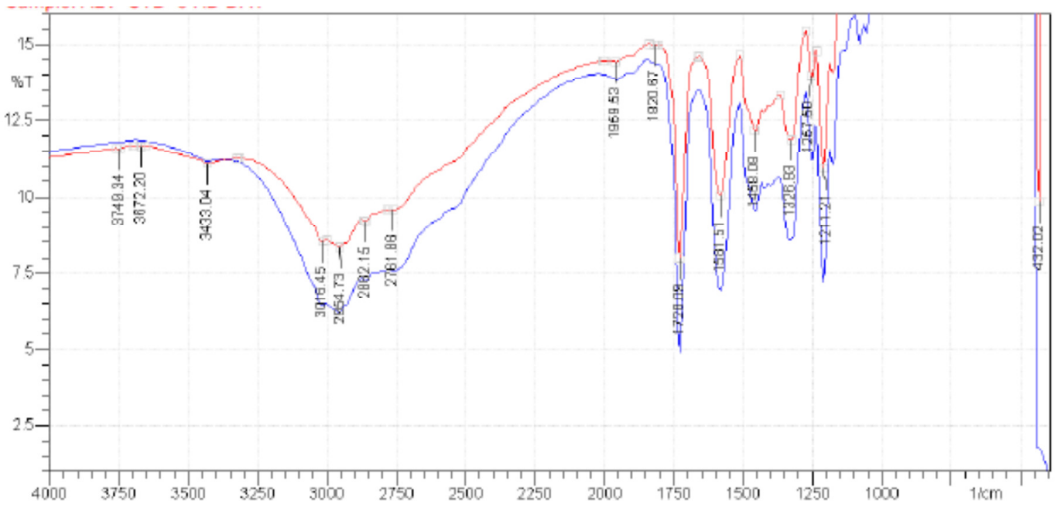


Fig: 24 Overlay IR Spectrum of Standard with Bulk 5th Day

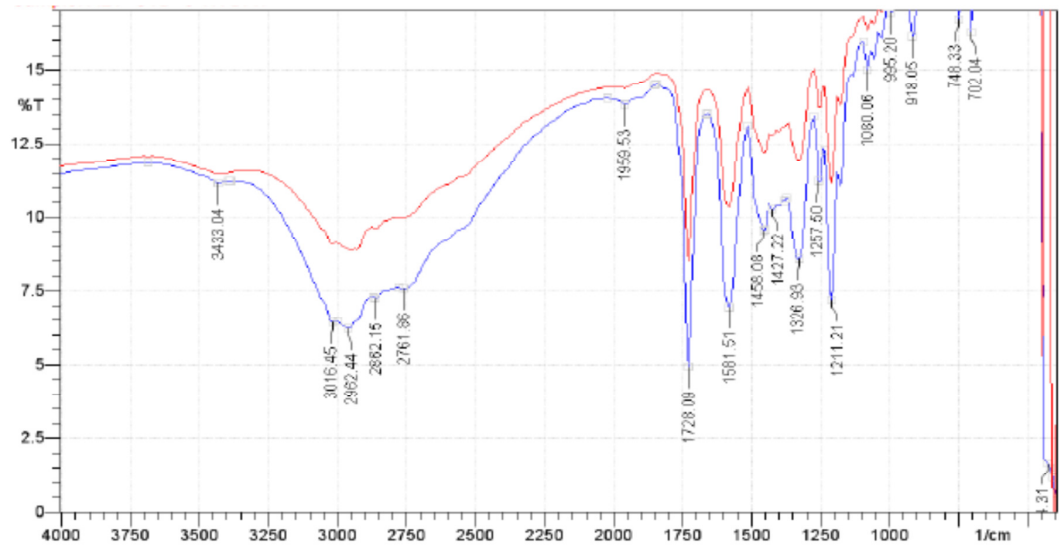


Fig: 25 Overlay IR Spectrum of Standard with Sample in 1st Day

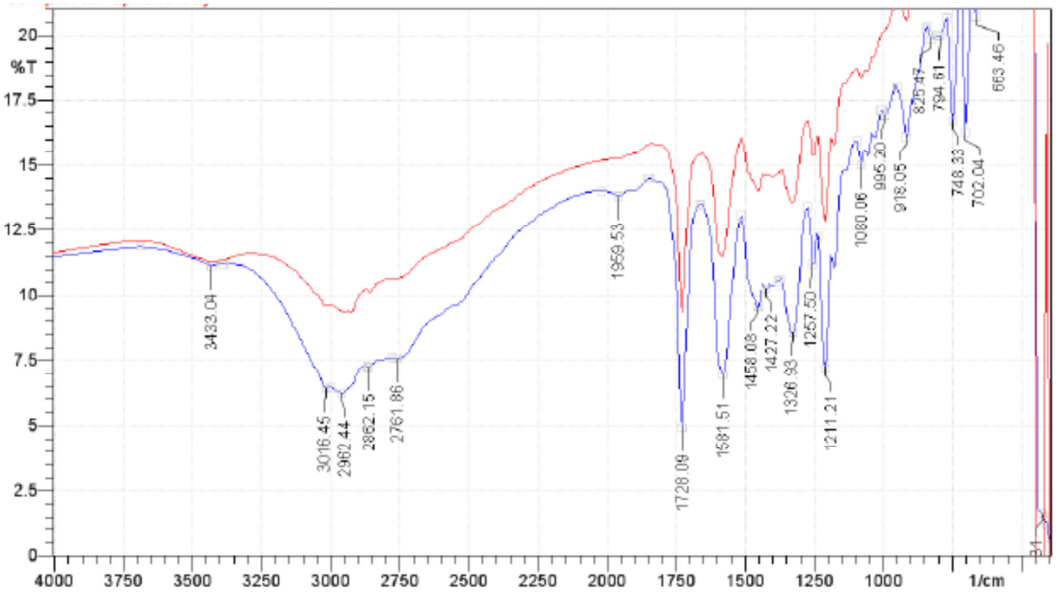


Fig: 26 Overlay IR Spectrum of Standard with Sample in 5th Day

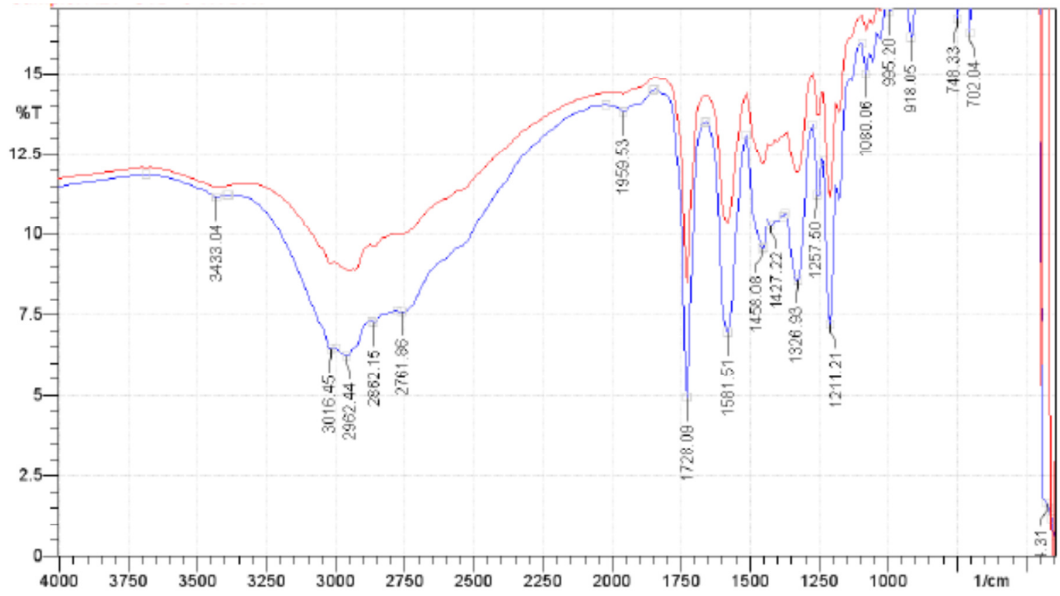


Table: 14

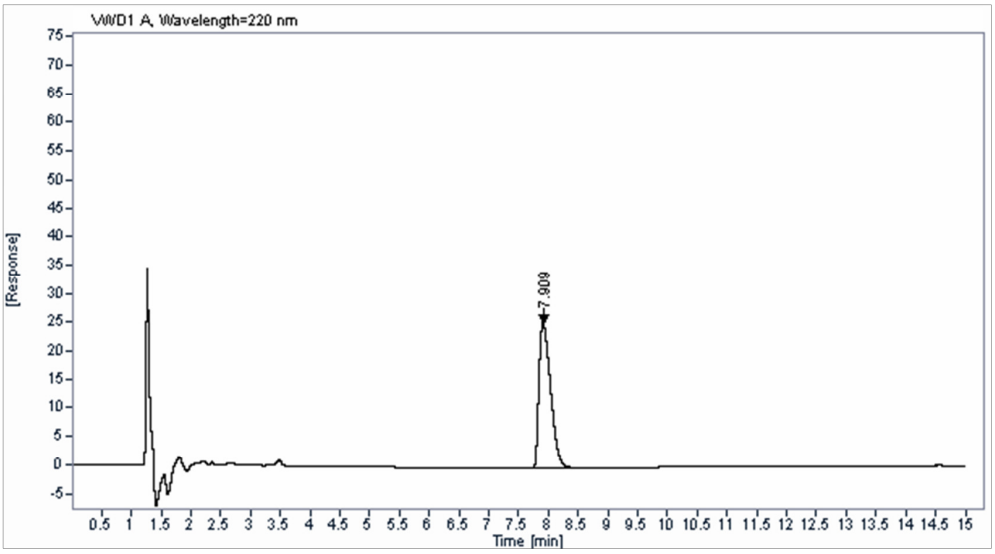
BAND FREQUENCY (cm^{-1})	BULK	SAMPLE	RESULT
3016	C-H Stretching Aromatic	C-H Stretching Aromatic	No changes observed
2962	C-H Stretching Alkane	C-H Stretching Alkane	No changes observed
1458	C=C Stretching Aromatic	C=C Stretching Aromatic	No changes observed
1211	C-C Stretching Alkane	C-C Stretching Alkane	No changes observed

IR spectroscopy study was performed for interday degradation study. The study reveals that there are no changes in the functional group present in the Alverine citrate bulk and formulation by KBr pellet technique.

3) RP- HPLC STUDY OF BULK&SAMPLE-UNDER STRESS CONDITIONS:

In RP- HPLC C-18 silica column was used for Alverine citrate assay. The mobile phase used in this assay was Acetonitrile and Sodium Lauryl Sulphate in the ratio of (1:1). 1ml/minute flow rate was maintained. The retention time of Alverine citrate was found to be 7.9. Chromatogram of Alverine citrate is given Fig.27

Fig: 27 CHROMATOGRAM OF ALVERINE CITRATE



INTRA DAY STUDY

Fig: 28 Chromatogram of Alverine citrate Bulk in 0.1N HCl (90mins)

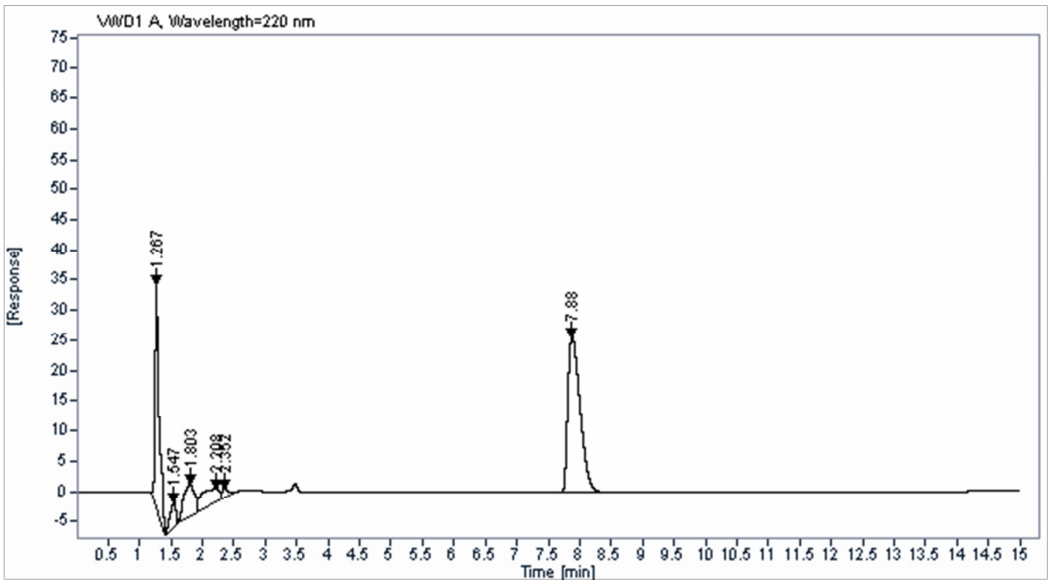


Fig: 29 Chromatogram of Alverine citrate Sample in 0.1N HCl (90mins)

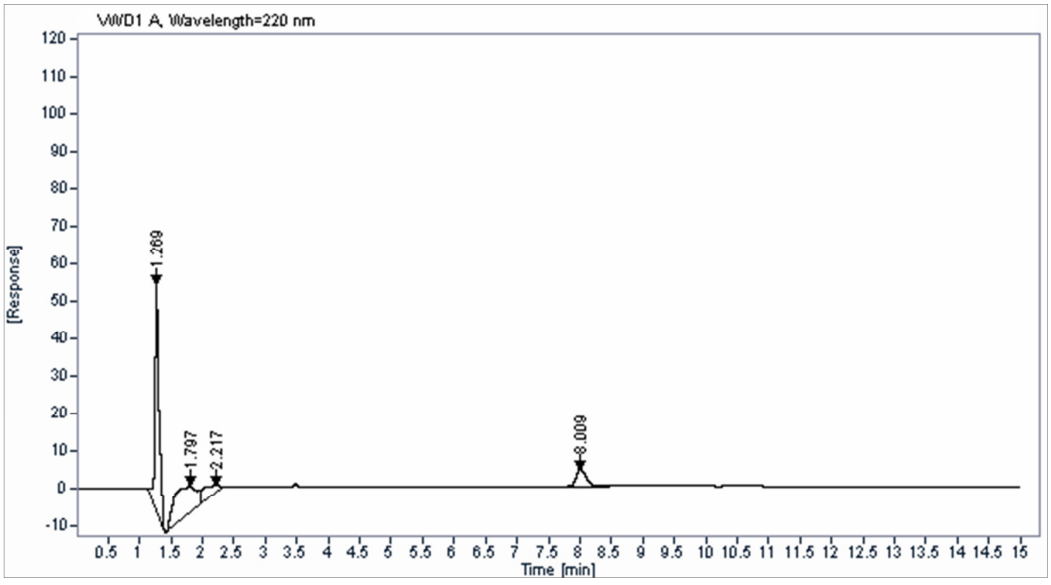


Fig: 30 Chromatogram of Alverine citrate Bulk in 30% H_2O_2 (90mins)

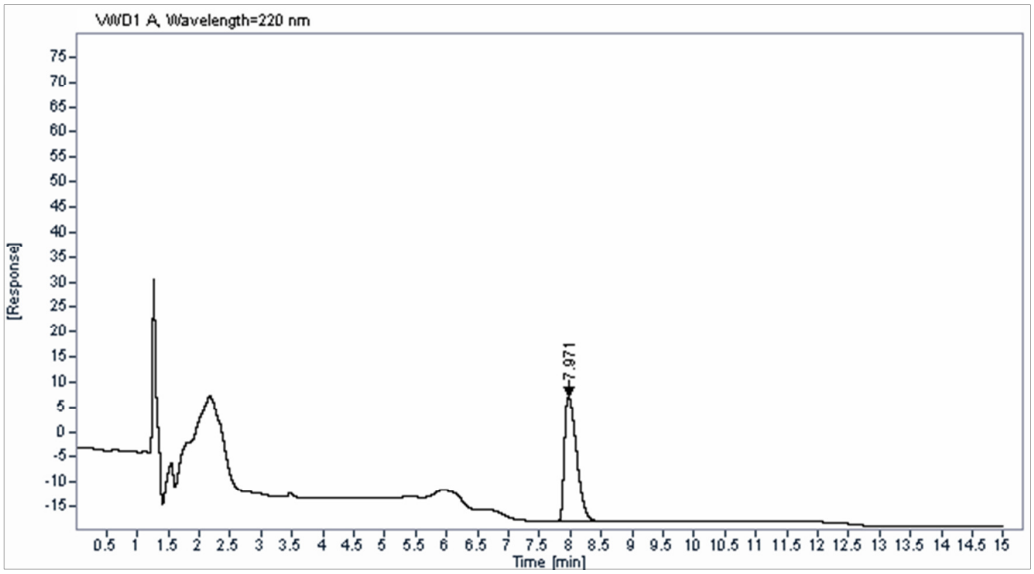
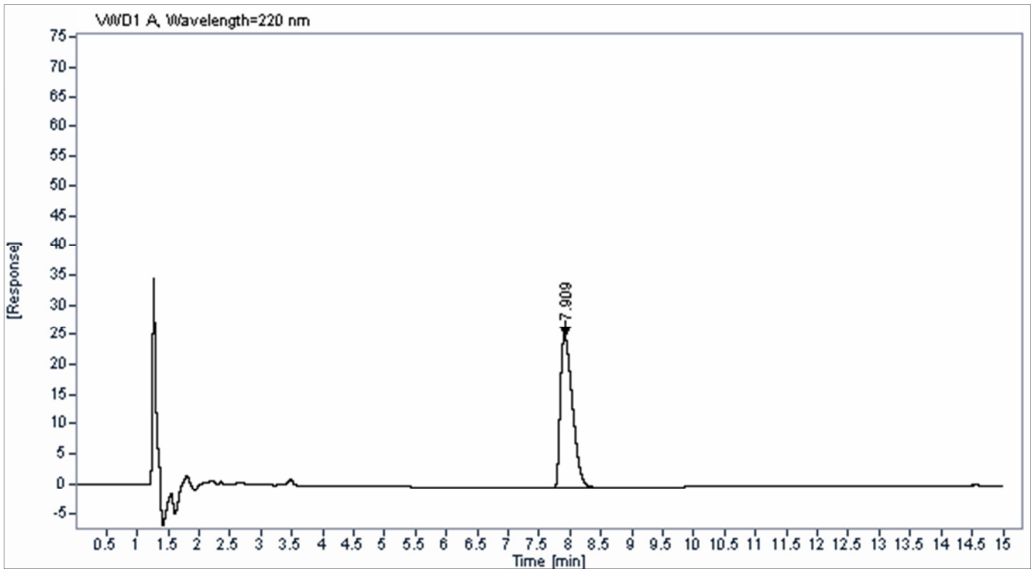


Fig: 31 Chromatogram of Alverine citrate Sample in 30% H_2O_2 (90mins)



3.1) Intraday Study Results For Degradation (Table: 15)

Stress condition	Time	Bulk Peak Area	Sample Peak Area
0.1N HCl(Acid)	90mins	169	219
30% H_2O_2(Oxidation)	90mins	225	234

Stress condition	Time	Bulk Percentage Content (%)	Sample percentage Content (%)
0.1N HCl(Acid)	90mins	50.1	65.0
30% H_2O_2 (Oxidation)	90mins	66.6	69.4

RP–HPLC study of Intraday degradation shows that moderate amount of degradation has occurred. Compared to oxidative degradation acid degradation was more prominent in the sample.

INTERDAY STUDY

Fig: 32 Chromatogram of Alverine citrate Bulk in 0.1N HCl (3rd day)

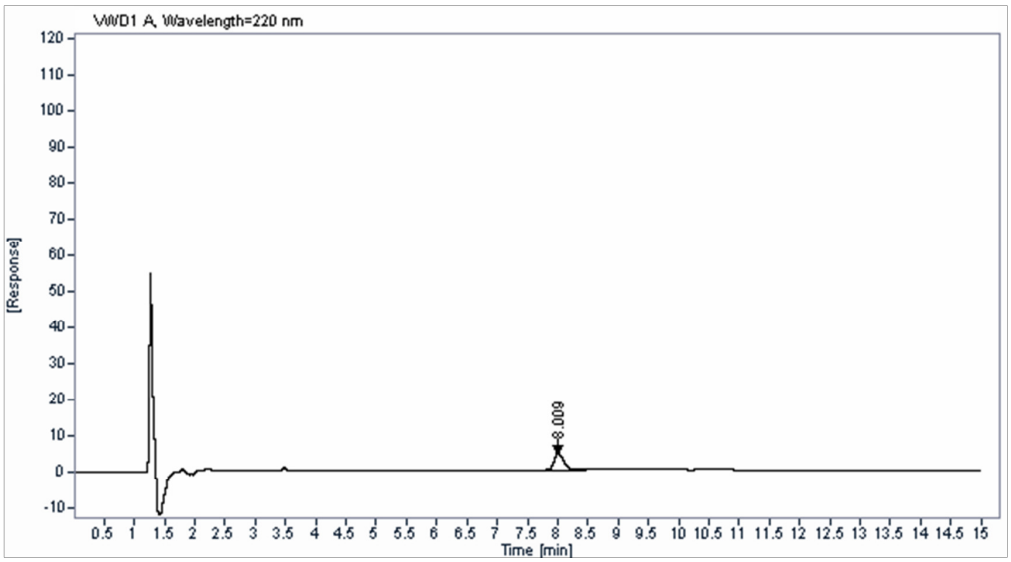


Fig: 33 Chromatogram of Alverine citrate Sample in 0.1N HCl (3rd day)

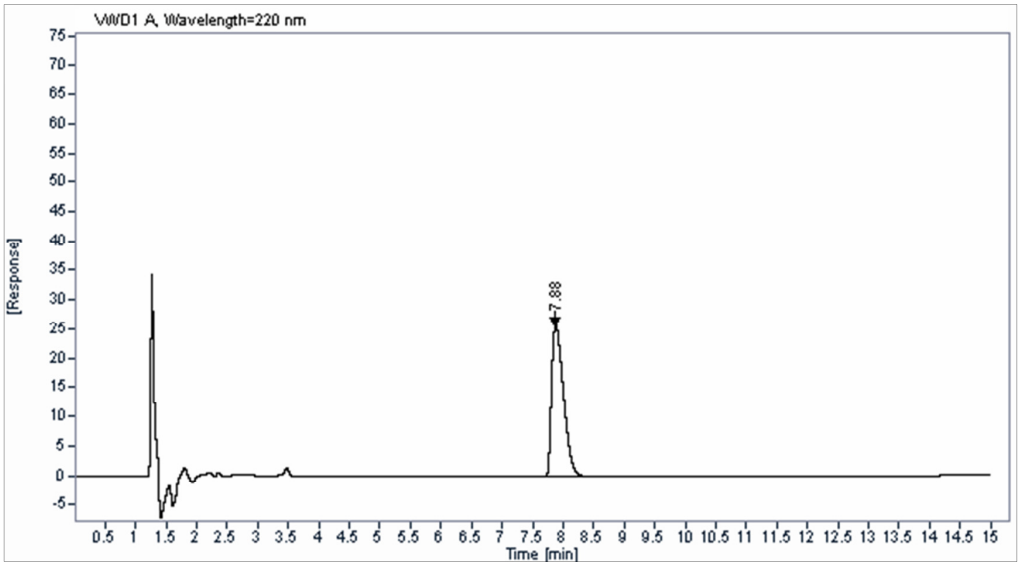


Fig: 34 Chromatogram of Alverine citrate Bulk in 30% H_2O_2 (3rd day)

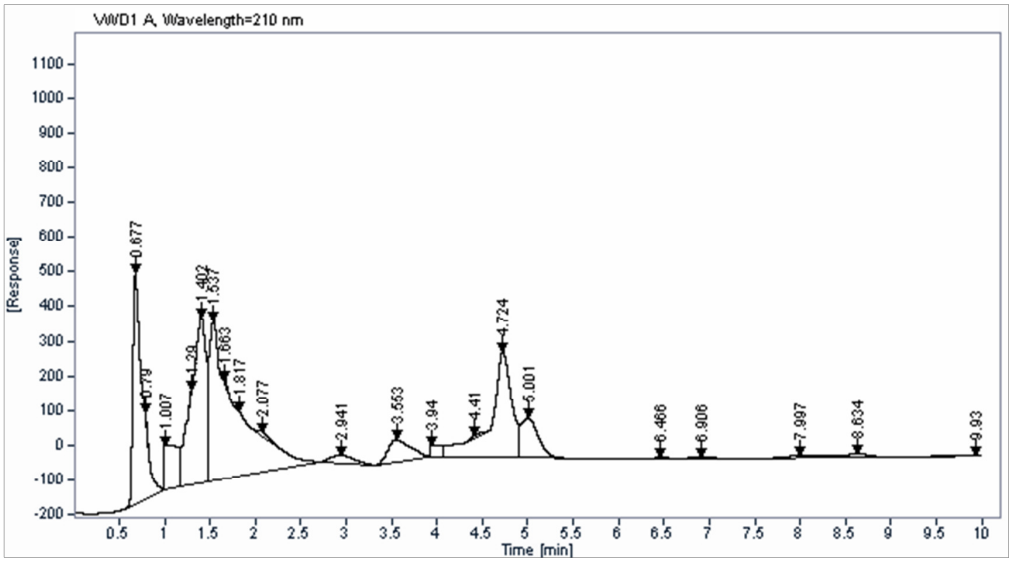


Fig: 35 Chromatogram of Alverine citrate Sample in 30% H_2O_2 (3rd day)

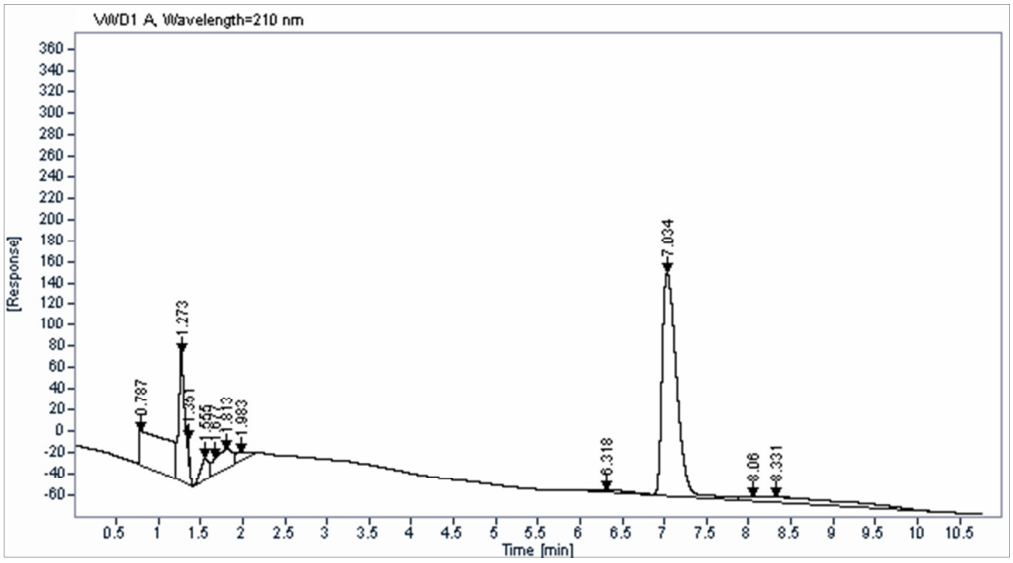


Fig: 36 Chromatogram of Alverine citrate Bulk (Thermal 3rd day)

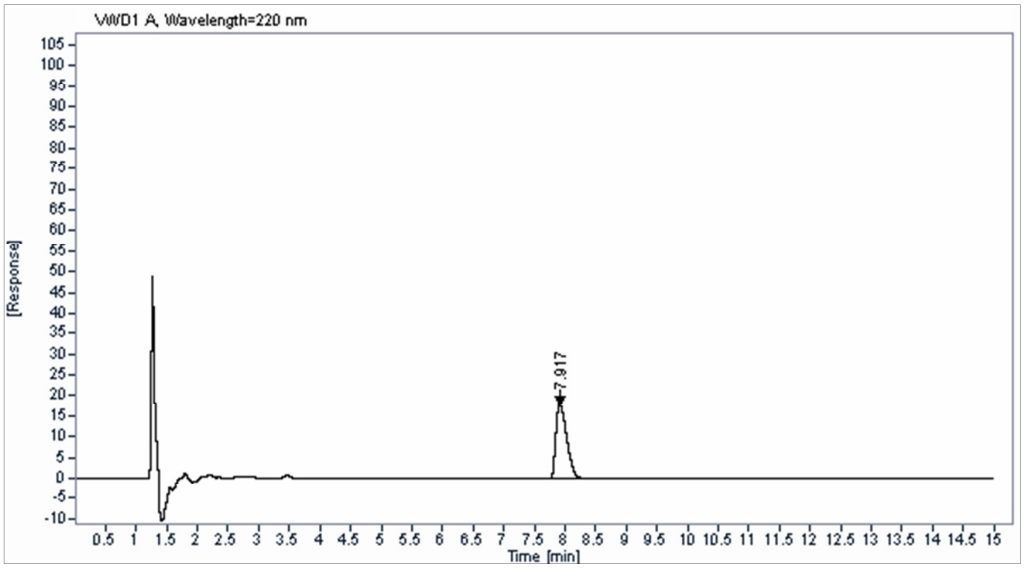


Fig: 37 Chromatogram of Alverine citrate Sample (Thermal 3rd day)

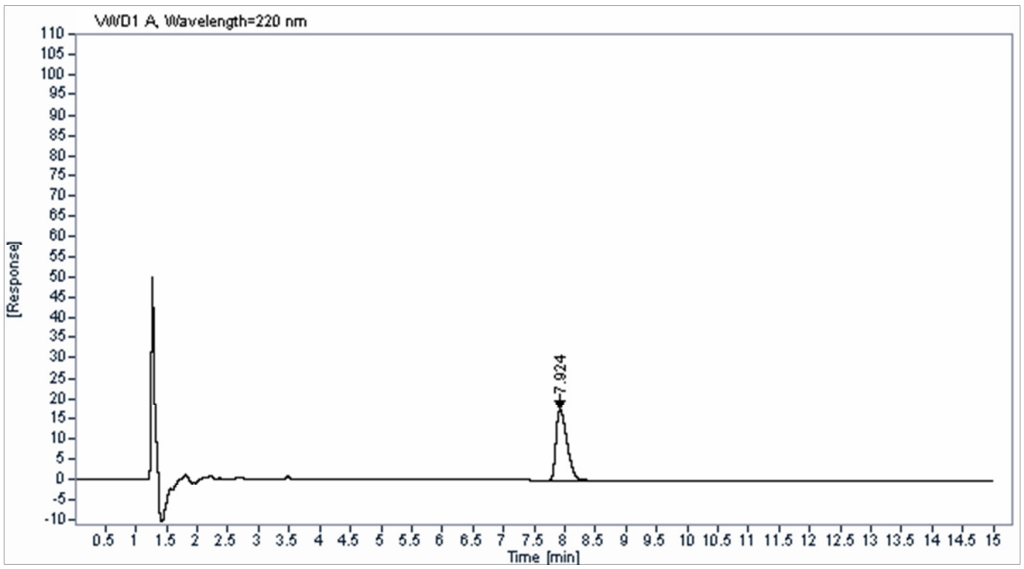


Fig: 38 Chromatogram of Alverine citrate Bulk (Sun light 3rd day)

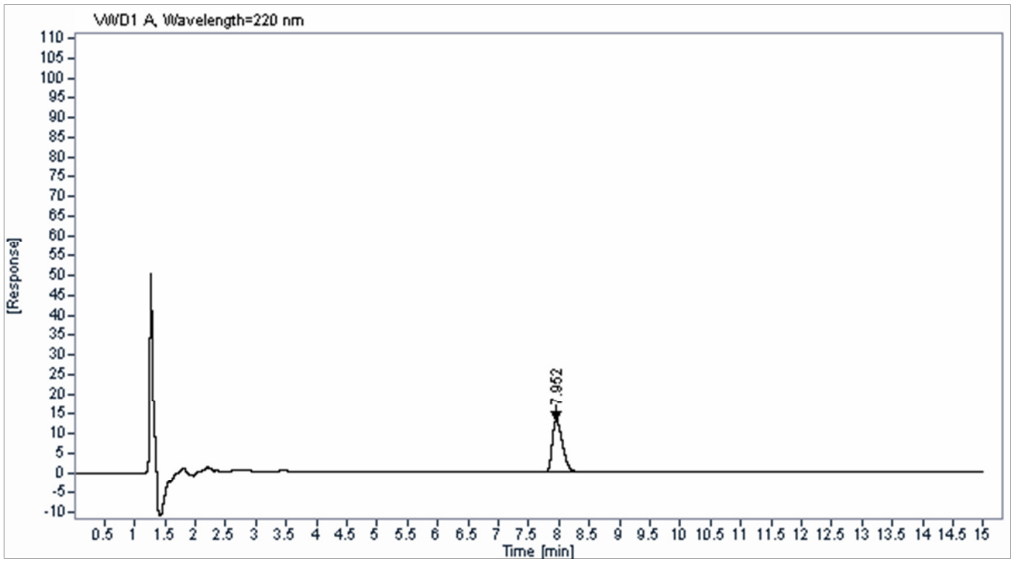
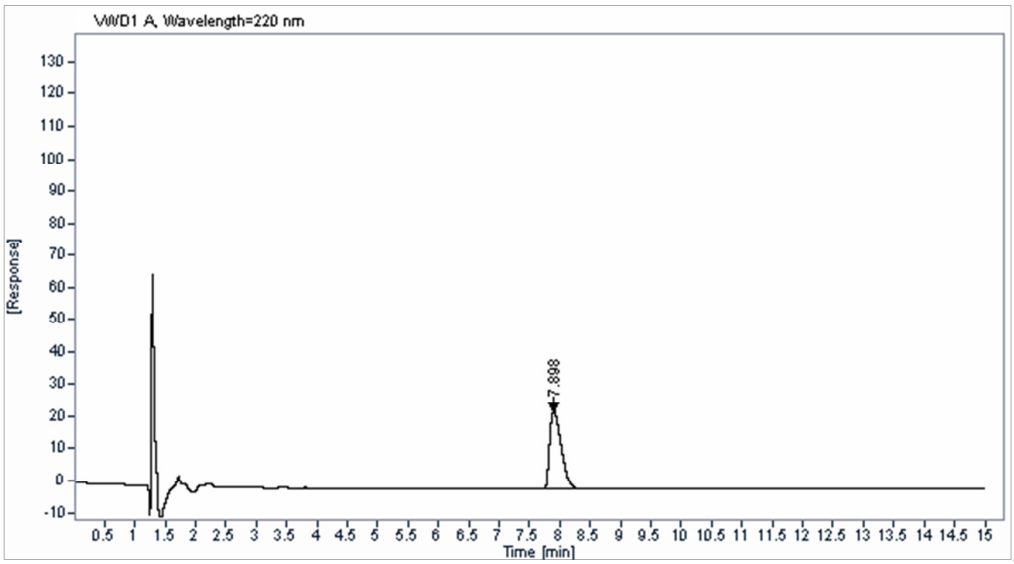


Fig: 39 Chromatogram of Alverine citrate Sample (Sun light 3rd day)



Interday Study Results for Degradation (Table: 16)

Stress condition	Time	Bulk Peak Area	Sample Peak Area
0.1N HCl(Acid)	3rd Day	65	240
30% H_2O_2(Oxidation)	3rd Day	-	-
Thermal (50ºC)	3rd Day	188	199
Sunlight (Photolysis)	3rd Day	250	238

Stress condition	Time	Bulk Percentage Content (%)	Bulk Percentage Content (%)
0.1N HCl(Acid)	3rd Day	16.8	71.2
30% H_2O_2(Oxidation)	3rd Day	-	-
Thermal (50ºC)	3rd Day	55.7	59.0
Sunlight (Photolysis)	3rd Day	74.1	70.6

Interday degradation study in HPLC shows that the more amount of degradation was seen in bulk compared to standard. Complete degradation was observed in Interday oxidative degradation. Extensive degradation was observed in acid hydrolysis. Thermal and Photolytic degradation showed moderate amount of degradation. In Thermal bulk has undergone more amount of degradation. In Photolytic degradation sample has undergone more amount of degradation.

4. THIN LAYER CHROMATOGRAPHY

TLC is used for identification of degraded products. The mobile phase used for degradation of Alverine citrate was found to be Chloroform and methanol in the ratio of 9:1. Various mobile phases were tried for Alverine citrate .The results of intraday and interday are given Table17 and 18 respectively.

4.1. Intraday study results (Table: 17)

STRESS CONDITION	SAMPLE TYPE	NO OF SPOTS	SECONDARY SPOTS	Rf VALUE
Acid Hydrolysis 0.1N HCl	Bulk	1	0	0.52
	Sample	1	0	0.53
Oxidative Degradation 30% H_2O_2	Bulk	1	0	0.55
	Sample	1	0	0.53

4.2. Interday Degradation Study results. (Table: 18)

STRESS CONDITION	SAMPLE TYPE	NO OF SPOTS	SECONDARY SPOTS	Rf VALUE
Acid Hydrolysis 0.1N HCl	Bulk	1	0	0.41
	Sample	1	0	0.46
Oxidative Degradation 30% H_2O_2	Bulk	0	0	0
	Sample	0	0	0
Thermal Degradation	Bulk	1	0	0.45
	Sample	1	0	0.42
Photolytic Degradation	Bulk	1	0	0.48
	Sample	1	0	0.49

Intraday TLC study shows that there is no secondary spot and shows the amount of degradation. Rf values of bulk and sample were found to be 0.52 and 0.53 respectively for intraday acid degradation. In interday oxidative degradation no spot was observed in standard and sample. This indicates that complete degradation of Alverine citrate standard and sample.

SUMMARY AND CONCLUSION

The present work entitled as **"FORCED DEGRADATION STUDIES OF ALVERINE CITRATE IN BULK AND FORMULATION BY UV, IR SPECTROPHOTOMETRY, TLC, AND RP-HPLC METHOD"** is a study about the stability of Alverine citrate in various conditions.

The study comprises of

- Alkali hydrolysis, Oxidative degradation, and Thermal and Photolytic degradation were performed.
- Degraded samples were quantified by UV spectroscopy and HPLC.
- |The results of bulk and sample compared with standard.
- Degraded samples were identified by TLC.
- Functional group changes in degraded samples were identified by IR.

Every procedure was repeated three times. Various stress conditions were tried to study the stability of Alverine citrate. The stress conditions used are Alkali, Acid hydrolysis, Oxidative degradation, Thermal and Photolytic degradation.

The results of the studies are

In intraday hydrolytic degradation, bulk underwent more degradation compared to sample from 30mins to 90mins study.

In interday acid degradation, the assay values of bulk and sample were found to be 15.1% and 56.0% respectively, at the end of 5^{th} day degradation.

Complete degradation was observed in interday oxidative degradation. It shows that Alverine citrate is vulnerable to oxidative condition.

Moderate amount of degradation was observed in both Photolytic and Thermal degradation.

IR spectrum shows that no functional group changes were observed in both Photolytic and Thermal degradation.

HPLC chromatogram shows that formation of impurities in intraday oxidative degradation.

Interday oxidative degradation chromatogram shows complete degradation and more amounts of degradation products.

No impurity peak was observed in both Photolytic and Thermal degradation chromatogram.

No secondary spots were observed in TLC but difference in Rf value was observed.

All the observations show Alverine citrate is vulnerable to Alkali hydrolysis, Oxidation, Thermal and Photolytic degradation.

The future scope of study includes the determination of degradation pathways of drug substance and drug product. Identification of degraded products in formulation that are related to drug substance versus those that are related to non-drug substance (Additives, Excipients). Structural elucidation of degraded fragment and determination of intrinsic stability of drug substances.

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